"A STUDY ON UTILITY OF CARTRIDGE-BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT) IN EARLY DETECTION OF PULMONARY TUBERCULOSIS IN SPUTUM NEGATIVE RETROVIRAL POSITIVE PATIENTS"

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ABSTRACT

Introduction:

Tuberculosis is the most common opportunistic infection amongst people living with HIV. HIV-TB co-infection further increases the mortality of an individual. The clinical presentation of pulmonary tuberculosis depends on the immune status of an individual and an immune-suppressed individual with HIV can present with atypical features, thus posing diagnostic challenges. For the detection of TB using microscopy, the sensitivity is an issue and it is addressed partly by the implementation of CBNAAT in HIV patients for the detection of TB.

Aims and objectives:

To assess the usefulness of CBNAAT in the early detection of pulmonary tuberculosis with smear negative HIV patients and its correlation with CD4 count.

Materials and methods:

PLHIV patients, age more than 18 years with symptoms suggestive of PTB were considered. All patients underwent detailed history and clinical examination. All patient's latest CD4 counts were noted. Total of 94 PLHIV patients with sputum microscopy negative samples who met the inclusion criteria were included in the study. Sputum sample of at least 1 ml of each patient was collected in a sterile falcon container was analysed by CBNAAT Xpert MTB/RIF. Data analysis was done using MS excel software. Diagnostic yield of CBNAAT was compared with CD4 counts of patient's.

Observations and results:

Total 94 PLHIV patients with presumptive PTB with sputum microscopy negative were included in the study. Among 94 PLHIV patients, 51 were females and 43 were males. Mean CD4 count was 273. Out of 94 sputum negative samples, CBNAAT detected 13(13.8%) PTB cases, which was significant. One rifampicin resistance case was detected among 13 CBNAAT positive cases. One PTB cases detected in CD4 count up to 100cells/ml. 5 cases between CD4 count 100-200, 2 cases between CD4 count 201-300, 3 cases between CD4 count 301-400 and 2 cases detected in CD4 count more than 500.

Conclusion:

CBNAAT is to be indicated as a primary diagnostic test in PLHIV with presumptive TB.CBNAAT detects pulmonary TB in PLHIV with greater efficacy than sputum microscopy, also helping in early diagnosis in less than 2 hours. It also detects rifampicin resistance with high specificity and can be used for screening for MDR-TB so that early therapy can be started, thus decreasing the incidence of MDRTB.

Keywords:

HIV-TB co-infection, sputum microscopy, CBNAAT, CD4 count

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LIST OF ABBREVIATIONS USED

AFB	:	Acid Fast Bacilli
AIDS	:	Acquired Immuno Defeciency Syndrome
ART	:	Anti Retro Viral Therapy
ATT	:	Anti Tuberculous Therapy
BMI	:	Body Mass Index
CBNAAT	:	Catridge Based Nucleic Acid Amplification Test
CD 4	:	Cluster Of Differentiation
CFU	:	Colony Forming Unit
CMI	:	Cell Mediated Immunity
CNS	:	Central Nervous System
CT	:	Computerised Tomography
EPTB	:	Extra Pulmonary Tuberculosis
FNAC	:	Fine Needle Aspiration Cytology
HIV	:	Human Immuno Deficiency Virus
IL	:	Interleukin
INH	:	Isoniazid
LED	:	Light Emitting Diode
MDR	:	Multi Drug Resistant
MRI	:	Magnetic Resonance Imaging
MTB	:	Mycobacterium Tuberculosis
MTB/RIF	:	Mycobacterium Tuberculosis/Rifampicin Resistance
PA view	:	Postero Anterior
PLWHA	:	Persons Living With Hiv And Aids
PTB	:	Pulmonary Tuberculosis
RIF	:	Rifampicin
RNTCP	:	Revised National Tuberculosis Control Program
RpoB	:	RNA Polymerase B
SPSS	:	Statistical Practice in Social Sciences
TB	:	Tuberculosis
TH 1	:	T Helper Cells
TNF	:	Tumour Necrosis Factor
TST	:	Tuberculin Sensitivity Test
XDR TB	:	Xtremely Drug Resistant Tuberculosis
ZN	:	Zeihl Neelson

INTRODUCTION

Tuberculosis is an infectious disease caused by a bacterium called Mycobacterium tuberculosis. Tuberculosis mostly involves the lungs; however, it can also affect other organs in the body[1].

According to WHO at least one third of 35.3 million people living with HIV worldwide are infected with latent TB. Globally about 14.8% of patients with TB are co infected with HIV. Persons co-infected with HIV-TB are 29.6 times (27.1 - 32.1) more likely to develop active TB disease than persons without HIV[2].

Infections amongst people with HIV Tuberculosis is one of the most common opportunistic and TB-HIV co-infections even further increases the mortality of an individual[3].

The clinical presentation of pulmonary tuberculosis depends on the immune status of an individual and an immune-suppressed individual with HIV can present with atypical features, thus posing diagnosticchallenges[4].

Detection of pulmonary tuberculosis by sputum-based techniques includes microscopy and culture. However, in people living with HIV, sputum production is scanty and the also the sputum contains a smaller number of bacilli due to fewer cavitation's, thereby decreasing the sensitivity and specificity of sputum microscopy as a diagnostic tool[4].

To overcome these shortcomings, mycobacterial culture is an alternative. It is a time-consuming technique which can take 4-8 weeks for the result thereby causing a delay in early initiation of anti-tubercular drugs, increasing the risk of transmission to close contacts and also the spread to extra-pulmonary regions within the same individual[5].

Clinical presentation and clinical forms of TB in HIV patients partly depends on CD4 counts. So, it is important to study impact of CD4 counts and development of tuberculosis in PLHIV[6].

Other opportunistic infections which have a selective range of CD4 counts in which the disease occurs, TB occurs throughout the course of HIV. The interaction between HIV and TB in persons with co-infection is bi-directional and synergistic. HIV infection is associated not only with an increased incidence of TB, but with altered clinical manifestations. While pulmonary TB can develop at any level of CD4 counts, extra pulmonary and disseminated forms of the disease is more common as immunodeficiency increases[7].

For the detection of TB using microscopy, the sensitivity is an issue and that's addressed partly by the implementation of CB NAAT in 2010 by WHO in HIV patients for the detection of TB. The X-pert MTB/RIF is a cartridge-based nucleic acid amplification test, an automated diagnostic test capable of identifying Mycobacterium tuberculosis DNA and rifampicin resistance by nucleic acid amplification in real time. CBNAAT can detect as few as 131 CFU/ml of MTB whereas sputum microscopy has a limit of detection of ~10,000 CFU/ml of MTB. X-pert MTB/RIF is the initial diagnostic test to detect pulmonary TB and rifampicin resistance in patients with signs and symptoms of tuberculosis as recommended by WHO[8].

Hence the present study will be considered.

AIMS & OBJECTIVES

To assess the usefulness of CBNAAT in the early detection of pulmonary tuberculosis with smear negative HIV positive cases and its correlation with CD4 count

REVIEW OF LITERATURE

Tuberculosis is an age-old infection caused by Mycobacterium Tuberculosis complex. It is thought to be emerged 70,000 years back in Africa and expanded to modern worlds then onwards[9]. This disease most commonly affects the lungs in two third of the patient but involves almost all of the organ systems in body and the systems other than lungs are involved in one third of the patients.

The public importance of this disease is that when it is drug susceptible and treated properly, treatable in majority of patients. If not treated, fatal in 50-60% of patients within 5 years[9].

EPIDEMIOLOGY

In the year 2013, 5.7 million new TB cases were reported worldwide to WHO. Out of which 95 percent was from the developing countries. But this is considered as around 2/3rd of total new cases as the infections are under diagnosed in the developing countries because of poor resources in detection as well as socio economic, cultural reasons prevailing mostly in the developing countries. Based on this, the estimated total new TB cases worldwide by WHO 2013 was 9 million.

Of the newly diagnosed total TB cases by WHO in 2013, 13% were HIV associated. And the mortality was also high in this group of patients. A total of 0.36 million out of Out of 1.49 million new patients 0.36 million patients were died in 2013 because of tuberculosis[9]. Coming to the matter of drug resistance, about 28% of the total cases of tuberculosis were drug resistant.

ETIOLOGICAL AGENT

Mycobacterium tuberculosis is a spore forming and rod-shaped bacteria, 0.5-3-micronlength. This is a gram negative, but acid-fast positive bacteria because of high content of mycolic acids, other long chain fatty acids and lipids. Some other

microorganisms also display some acid fastness. These include species of Nocardia, Rhodococcus, Legionella micdadei, and the protozoa Isospora & Cryptosporidium. In the cell wall of mycobacteria, lipids like mycolic acids are linked to underlying arabinogalactan and peptidoglycan molecules. Because of this structure, there is very low permeability of the cell wall to the anti-bacterial agents. Yet another molecule in the cell wall of mycobacteria, lipoarabinomannan, is involved in the interaction between bacteria and host cells, resulting in the increased survival of Mycobacterium tuberculosis inside the macrophages.

MODE OF TRANSMISSION

The transmission of infection is via aerosolized particles from respiratory tract when the patient coughs. These particles are less than 5-10 microns in diameter. These remain suspended in the atmosphere for several hours, and there are approximately 3000 such particles in one cough. On an average, a person may transmit infection to 20 contacts before being recognized as a patient. Transmission through skin or placenta are uncommon and not of much significance.

RISK FACTORS FOR ACTIVE ILLNESS

1. Recent infection with Mycobacterium tuberculosis
2. Fibrotic lesions, silicosis.
3. HIV co-infection
4. CKD on dialysis
5. Diabetes mellitus
6. Smoking
7. IV drug abuse, immune suppressive treatment
8. Malnutrition and underweight.

Among these the highest odd's ratios is for HIV infection. The relation with age is that the adolescent and early adulthood age groups are particularly more prone for active infection because of unknown reasons. In female, clusters in 25-34 years of age and there is a rise of active disease in elderly because of waning immunity.

NATURAL HISTORY

1. One third of PTB died within 1 year[9].
2. More than 50% died within 5 years.
3. 5-year mortality rate among sputum positive PTB 65%.

PATHOGENESIS AND IMMUNITY[10]

The infection with Mycobacterium tuberculosis begins with the inhalation of droplet nuclei containing large number of mycobacteria, released when an infectious patient coughs and when they are taken up by inhalation by a close by stander... Most inhaled bacteria are trapped in the upper airway tract and are expelled by ciliated mucosal cells. But a small fraction (usually less than10%) will reach the alveoli. Alveoli have a unique immunoregulatory environment. The pulmonary alveolar

macrophages, which are the key immunoregulatory cell in alveoli, have not yet been activated to phagocytose the bacteria.

The adhesion of TB mycobacteria to macrophages results from binding of the bacterial cell wall to a large variety of cell-surface molecules expressed over the macrophages, including complement receptors, the mannose receptor, the immunoglobulin receptors, and some scavenger receptors. The complement activation enhances the phagocytosis. After phagocytosis, TB bacteria will get captured into the lysosomes to form endosomes. They will cause endosomal manipulations, i.e., maturation arrest, lack of acid pH and ineffective phagolysosome formation. Because of these, they multiply inside the phagosomes such as phagosome lysosome fusion and inflammatory cytokine production. After phagosome formation, the survival of Mycobacterium.

Tuberculosis seems to be dependent on reduced acidification. This results in a lack of assembly of a complete vesicular proton adenosine triphosphate. A series of complex events are generated by the bacterial cell wall lipoglycan lipoarabinomannan (LAM). This inhibits the intracellular release of calcium. By this the Calcium Calmodulin pathway is impaired, and the TB bacteria survive within the phagosomes.

The phagosome is found to inhibit the production of phosphatidyl inositol 3 phosphate (PIP3). PIP3 causes membrane maturation and sorting in phagosomes, thereby the phagolysosome formation which would destroy the bacteria. If the bacilli successfully in arrest phagosome maturation, then the replication begins. Eventually the macrophage ruptures and releases its bacillary contents. Other uninfected macrophages are then recruited, and the infection cycle continues, ingesting dying macrophages and their bacillary content, thus in turn becoming infected themselves and expanding the infection.

After 3 weeks of initial infection, processed TB bacterial antigens reach the draining lymph nodes, and are presented by dendritic cells via MHCII to CD4 T cells. The IL 12 secreted by macrophages causes proliferation of CD4 T cells of Th-1 variety, and they secrete IFN Gamma. Interferon gamma causes macrophage activation-TNF secretion. Monocyte differentiation to epithelioid histolytic and granulomatous reaction. Also, iNOS is activated, NO acts as powerful oxidizer and release of reactive free radicals Nitrogen and Oxygen species.

CLINICAL MANIFESTATIONS

Pulmonary TB manifestations are broadly divided as pulmonary and extra pulmonary. Pulmonary tuberculosis diseases are categorized as primary and post primary. Primary TB occurs after the initial infection, affects mainly children, infectivity is low, cavitation's and permanent sequel are rare and affects middle and lower lung zones. Post primary/adult type affects mostly the apical and posterior segments and runs a chronic debilitating course. Extra pulmonary TB mostly manifests in lymph nodes, pleura, genital and urinary tract, joints and bones, meninges, peritoneum, and pericardium.

Lymph node disease manifest as painless matted nodes mostly in posterior cervical region. Associated pulmonary tuberculosis is present in less than 50% of patients. Lymph node tuberculosis is particularly frequent among children and HIV infected patients. Earlier, the tuberculous lymphadenitis was mostly caused by Mycobacterium bovid, but now it caused mostly by Mycobacterium tuberculosis.

The most common sites for tuberculous lymphadenopathy is posterior cervical group and supraclavicular groups. The painless swellings of lymph nodes in these regions were historically referred as scrofula. In early disease lymph nodes are discrete and over the time they progress to matted nodes. On passage of time, they

may develop fistulous tracts with discharge of caseous material. The systemic symptoms are usually uncommon, but present in HIV individuals.

The diagnosis in tuberculous lymphadenopathy can be established by fine-needle aspiration biopsy (FNAB) or surgical excision biopsy. The yield by fine needle aspiration is around 80 %, detection and with culture also yields around 90%, Lymph node involvement in HIV-TB, they have less organized granulomas, so more chance of detection by microscopy and culture. 20% of patients, there is pleural involvement. It's mostly due to hypersensitivity reactions to the TB microbial antigens or sometimes contiguous spread. Associated parenchymal lesions are seen in less than one third of patients. Pleural fluid CB NAAT is not generally recommended because of low sensitivity. Pleural biopsy is the preferred method of investigation.

Isolated pleural effusions are usually due to primary infection and hypersensitivity reaction to mycobacterial antigens. Post primary infections may result in contiguous spread if pleurisy is present. Depending up on the extent of reactivation, the effusion can be small, and resolve spontaneously. It may be sometimes large to cause symptoms like fever, pleuritic chest pain, and breathlessness. Physical examination reveals dullness to percussion and absence of breath sounds. A chest X ray reveals the effusion. In about one-third of cases, shows a parenchymal lesion. Pleural fluid aspiration and analysis is essential for reaching the diagnosis. Mostly the fluid is straw colored, and sometimes hemorrhagic. The nature of fluid is exudative with protein concentration of more than 50% of in serum. There will be normal to low concentration of glucose. pH will be normal to acidic, around 7.3. Total leukocyte count is usually 500–6000/μl). In early stages neutrophils are predominant, and in late stages lymphocytes are more predominant.

AFB are rarely seen on smear, and cultures often may be false negative sometimes. Determination of adenosine deaminase (ADA) in pleural fluid may be a useful screening test. If the value is too low TB may be excluded. Measurement of IFN-γ can be helpful. These are done either directly or through stimulation of T cells sensitized with mycobacterial antigens. Pleural biopsy is recommended over pleural fluid it reveals granulomas and/or yields a positive culture.

Genito urinary TB accounts for 10-15% of total tuberculous patients, and 75% will be having pulmonary involvement. Local symptoms like nocturnal, dysuria, frequency predominates. Routine urine investigations reveal pyuria, hematuria, and culture negative UTI. Lower urinary tract TB can progress to pyelonephritis, in kidneys etc. Genital TB is more common in females and affects fallopian tubes, endometrium more commonly resulting in infertility and abnormal menstrual bleeds. In males mostly present as epididimo-orchiditis, Bones and joints tuberculosis is about 10%.

CNS involvement is for about 5% of patients. Usually in younger age group's culture is the gold standard, detects up to 80%, but CB-NAAT also has a similar detection rate. Gastrointestinal TB is rare-3.5%. It more commonly involves terminal ileum and cecum and peritoneum. In TB peritonitis, yield by direct smear and culture of fluid is low. Peritoneal biopsy should be considered for detection.

HIV ASSOCIATED TUBERCULOSIS

Tuberculosis is the most common disease affecting HIV patients. Certain data highlighting the role of importance of HIV TB co infections are given below[8].

Tuberculosis is responsible for about 24% of all-cause mortality in HIV patients. In patients positive for Tuberculin sensitivity test (TST), the annual risk for

tuberculosis is 3-13%. Evolution of active tuberculosis is faster in patients with HIV, rather in a week as opposed to months to years in general population.

When Cell mediated immunity (CMI) is only partly affected, pulmonary TB is manifested as in immune patients, which is with infiltrates and cavities and less lymph node enlargements, whereas if severely affected, there will be diffuse infiltrates without cavitation is seen and there will be more lymph node enlargements, the disease resembles primary tuberculosis. Sputum samples are less frequently positive among HIV-TB co infections, so that there is a diagnostic challenge for these patients. Incidence of extra pulmonary tuberculosis is higher than the normal population, constituting about 40-60% of the cases. It is now recommended that the X-pert MTB/RIF assay should be used for the diagnosis of TB in HIV, not the microscopy. Treatment should not be delayed if Expert MTB/RIF assay is positive. But culture is the gold standard test.

TB-HIV co-infection is associated with poor prognosis than when either disease present alone. HIV infection predisposes the patient to severe forms of TB such as disseminated TB and drug resistant TB. Tuberculosis in turn increases the viral load leading to higher mortality and morbidity in that patient[11]. Therefore, it is important to diagnose and treat TB early in those patients. Decreased CD4 counts are used as marker for suspicion of severe forms of tuberculosis. CD4 cells are the T-lymphocytes, which are involved in cell mediated immunity. These cells help to coordinate the immune response by stimulating other immune cells such as macrophages, B lymphocytes and CD8 T lymphocytes which are mainly involved in fighting off infections.8 In HIV infection, HIV virus gains entry into CD4+ T cell via attachment to CD4 receptor and brings about conformational change in gp120 allowing HIV virus to bind to co-receptors expressed on host cell and enter the cell

membrane and merge with RNA resulting in replication of viral progeny. Hence CD4 counts reflect the level of immunity and risk for opportunistic infections can be acquired[7].

Yet another important consideration is the Immune reconstitution inflammatory syndrome (IRIS). This occurs in the HIV-TB co infected patients if we are starting ART first because of undiagnosed TB. When effective ART is implemented, CMI of the patient improves and there is a increased cytokine release to the tuberculosis bacilli, which will cause flare-up of symptoms and signs like lymphadenopathy, respiratory symptoms or chest X ray findings. Decreased baseline CD4 count and earlier starting of ART are the main risk factors. This is the reason we must rely upon a method of higher diagnostic yield to diagnose TB in HIV rather than the conventional methods. HIV patients with culture positive or AFB positive TB may present with normal chest radiograph. The X-pert MTB/RIF assay, CBNAAT assay is the rapid diagnostic test then. There is a sensitivity of around 60% among AFB-negative culture-positive cases and 97% among AFB positive cases.

WHOM TO TEST?

How the TB diagnosis should take place in India is guided by the standard tests by Revised National Tuberculosis Control of India (RNTCP). These standards were published in 2014 and describe the tests and protocol for the diagnosis of tuberculosis for all those patients who are suspected of tuberculosis including the special categories, like as those with tuberculosis and HIV co infection. Any individual who has signs and symptoms suggestive of tuberculosis including

1. Cough for more than 2 weeks.
2. Fever for more than 2 weeks.
3. Significant weight loss, loss of appetite.

4. Hemoptysis.
5. Abnormality in chest x-ray with respiratory symptoms.
6. Household contacts of pulmonary TB with respiratory symptoms.

WHOM TO SCREEN?

1. People living with HIV (PLHIV/PLWHA)
2. people who are malnourished
3. People who have diabetes or cancer
4. People on steroid therapy
5. People on immune suppressive therapy.

These populations should be regularly screened for signs and symptoms suggestive of tuberculosis.

WHAT IS ENHANCED CASE FINDING?

Having a high level of suspicion for tuberculosis in all medical encounters and then identifying or excluding tuberculosis using a combination of clinical questionnaires, radio graphical and simple microbiological testing. Enhanced case finding should be undertaken in certain high risk population groups like healthcare workers prisoners slum dwellers certain occupational groups such as mineworkers, Laboratory workers.

DIAGNOSIS OF TUBERCULOSIS

1. AFB MICROSCOPY
2. NUCLEIC ACID AMPLIFICATION TECHNOLOGY
3. MYCOBACTERIAL CULTURE
4. RADIOGRAPHIC PROCEDURES
5. DRUG SUSCEPTIBILITY TESTING
6. ADDITIONAL DIAGNOSTIC PROCEDURES

AFB MICROSCOPY

The most reliable single method in diagnosis of Tuberculosis is sputum microscopy. Direct or concentrated smears are used normally. It's recommended to use new slides for every use, i.e. the slides should never be reused. It should be prepared from thick purulent part of sputum. They are dried and heat fixed and stained, to be examined under microscope.

Zeihl-Neelson staining or fluorescent staining is used. If ZN staining, it's viewed under oil immersion objective, under light microscope, the bacteria are seen as bright red rods.

A minimum of 10,000 bacilli should be present in 1 milliliter of sputum for ready diagnosis by smear. To give a negative report, a minimum of 300 fields should be examined. And a positive report is given if at least two typical bacilli are demonstrated. Typical bacilli mean appearing barred or beaded, since saprophytes are stained uniformly.

TABLE 1: ZN SMEAR EVALUATION AND AFB REPORT

NO. OF AFB	NO OF FIELDS	REPORT
0	300	NOT SEEN/ NEGATIVE
1-2	300	DOUBTFUL/ REPEAT
1-9	100	1+
1-9	10	2+
1-9	1	3+
10 OR MORE	1	4+

When large number of smears is diagnosed daily, fluorescent microscopy is better. The smears are stained with fluorescent stains and visualized under ultraviolet illumination and appear as bright rods in contrast to a dark background. LED microscopes are also available which are cheaper and are as good as traditional fluorescent microscopes in sensitivity.

ADVANTAGE OF MICROSCOPY

1. Cost effective [12]
2. Maintenance cost is low

DISADVANTAGES

1. Time consuming
2. Inter observable variation
3. Less sensitive, poor positive predictive value[12]
4. Saprophytes can be mistaken for true positives
5. Biosafety issues
6. Not much suitable for specimens other than sputum
7. HIV-TB confection, smear deviation rate is low

NUCLEIC ACID AMPLIFICATION TECHNIQUES[9]

This is based on the amplification of nucleic acids of the Mycobacterium Tuberculosis, so that, the specificity is high.

ADVANTAGES

1. Less time consuming
2. Detection and Rifampicin resistance identified within 2-3 hours.
3. Minimal biosafety and training requirements[11].
4. Sensitivity of 98% in AFB positive and 70% in AFB negative.
5. Can be housed in non-conventional laboratory settings[11].

DISADVANTAGE

Maintenance charges are relatively higher.

The current WHO recommendation[13] is to use the nucleic acid amplification test as the initial diagnostic test in suspected MDR-TB adults and pediatrics HIV-associated TB. If resources available as a follow up test to smear positive TB CSF, from suspected TB meningitis. No respiratory specimens like gastric lavage, FNAC specimens, pleural biopsies etc. This test has a sensitivity of 98%.

CULTURE OF MYCOBACTERIA

Definitive diagnosis of tuberculosis is based on culture or nucleic acid amplification. Specimens are cultured on egg or agar medium (e.g., Lowenstein–Jensen (LJ) or Middlebrook) and incubated at 37°C. As the Mycobacteria are slow growers, 4–8 weeks are required for growth to be detected. M. tuberculosis is identified presumptively with growth time, pigmentation of colony, morphology, and biochemical tests for speciating the isolates. In well-equipped laboratories; liquid culture and molecular methods HPLC for isolation and species identification instead of solid culture and the biochemical tests.

Fluorescent growth indicator tubes are available for the rapid identification of growth now a day. Immunochromatographic low-cost antigen detection is also aiding in species identification. All these methods have limited the time needed for culture identification to be reduced to about 2-3 weeks. So other main disadvantages are requirement of sophisticated laboratory, Cost, Time Consumption.

ADVANTAGES

1. Gold standard for diagnosis
2. Drug sensitivity possible simultaneously.

DRUG SUSCEPTIBILITY TESTING

1. Direct on liquid medium-3 weeks
2. Indirectly with culture isolates on solid or liquid media –about 8 weeks.
3. X-pert MTB/RIF assay detects Rifampin resistance
4. Molecular line probe assays is available now for INH and Rifampicin
5. Microscopically observed drug susceptibility (MODS)-noncommercial, cheap
6. Nitrate reductase assays -useful in resource poor scenarios

RADIOLOGICAL DIAGNOSIS

The initial doubtfulness of pulmonary tuberculosis is based on chest X-ray. The upper lobe disease, pulmonary infiltrates and cavities are classical finding. In HIV-TB co infection, the chest X-ray findings are not seen as the classical picture, more when the immunity is low. CT Chest is helpful with doubtful X-rays and Extra Pulmonary Tuberculosis like Pott's spine. MRI is useful for TB meningitis.

SEROLOGICAL TESTS

They are based on antibody detection to Mycobacterial antigens, mostly used in developing countries. Disadvantages include Not useful for diagnosis, especially with low probability of TB, Low sensitivity, and specificity, Poor reproducibility[13]. WHO issued negative recommendation in 2011 for these tests even in resource poor settings[14]. Determinations of ADA and IFN-γ in pleural fluids are considered as adjunctive tests in pleural TB, not recommended in other extra pulmonary tuberculosis.

RNTCP LABORATORY NETWORKS

A wide network of laboratories was established by RNTCP throughout India, TB tests can be done to diagnose people who have TB. These are primarily intended for Proper diagnosis of TB with good sensitivity and specificity. For the assessment and follow up of detected tuberculosis and for the timely detection of drug resistance in TB patients.

The laboratory system includes:

1. National Reference Laboratories or (NRLs)
2. State level Intermediate Reference Laboratories or (IRLs)
3. Culture & Drug Susceptibility Testing laboratories (C and DST laboratories)
4. Designated Microscopy Centers or (DMCs).

LED FLOURESCENT MICROSCOPY

In light microscopy using ZN technique, each of the smear testing may take an average of 10-15 minutes. The workload for laboratories is high and may consequently result in decreased reliability. Fluorescent microscopy (FM) was introduced as an alternative to light microscopy.

The advantage over conventional microscopy includes 10% more sensitive than ZN smear microscopy[15]. Fluorescent AFB can be seen in a lower magnification, so they can be examined in lesser time compared to light microscopy. The time reduction is about 25%, can reduce the laboratory workloads. But the disadvantages include high cost and complexity of system as it is using mercury vapor lamps. There is a need for a dark room, and health risks to laboratory workers due to exposure of ultraviolet rays are yet another concern. Because of these technical difficulties, illumination systems based on Light emitting diodes (LED) were implemented.

LED fluorescent microscopy holds several advantages over the conventional mercury vapor lamp fluorescent microscopy as well as the standard light microscopy, comparatively in-expensive and have an effective lifespan of thousands of hours[16]. Easily manageable in terms of power supply, that is it can work even with power supply from batteries. Because of all these advantages LED fluorescent microscopy is more suitable for resource poor countries. One important concern is with specificity. Some of the studies showed that, LED fluorescent microscopy has higher sensitivity but lower specificity than ZN smear microscopy when compared to the gold standard, culture of Mycobacteria.

XPERT MTB/RIF ASSAY/CBNAAT-IN AND OUT

The X-pert MTB/RIF assay is a cartridge based nucleic acid amplification test, which is an automated test that can identify MTB DNA and resistance to Rifampicin (RIF) by nucleic acid amplification test or NAAT. It was developed by the laboratory of Professor David Aland at the University of Medicine and Dentistry of New Jersey[17].

In the month of December 2010, the world health organization endorsed the X-pert MTB/RIF assay in TB endemic countries[18]. Tuberculosis is still a deadly and fiery public health threat today, though it is considered as an age-old disease developing along with mankind evolution. Tuberculosis is mostly diagnosed by chest X-rays, the microscopy with special stains, the growth in culture and the Mantoux test. The smear microscopy test has some problems in HIV positive patients, children and in patients with low bacterial load.

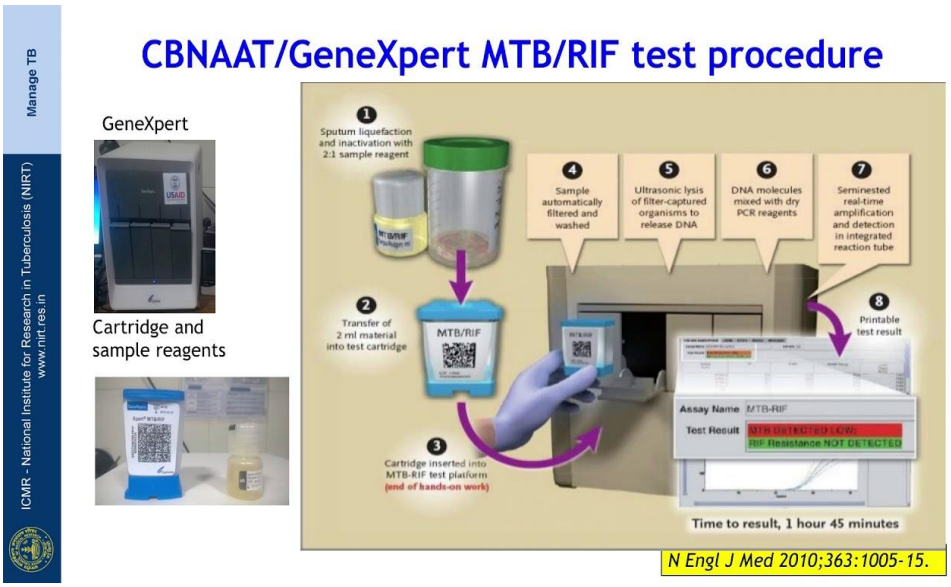
The X-pert MTB/RIF assay has high sensitivity & specificity for detection of pulmonary TB. An in vitro study demonstrated detection of 131 colony forming units (cfu) per milliliter compared to 10,000 cfu with conventional smear microscopy[19].

Susceptibility to drugs could only be diagnosed from the growth of MTB in culture which takes long time, about six weeks and needs high quality laboratories and cost. Drug susceptibility testing is highly relevant because tuberculosis is becoming increasingly resistant to two of the major anti-TB drugs, INH and Rifampicin, which is called MDR TB, and it needs longer time and special drugs to treat.

HOW IT WORKS?

The X-pert MTB/RIF assay purifies and concentrates Mycobacterium Bacilli from samples, isolates the genomic material from these captured bacteria by sonication and then amplifies the genomic DNA by polymerase chain reaction (PCR). This process also identifies all the clinically significant mutations in the RNA polymerase beta (rpoB) gene causing Rifampicin resistance in the Mycobacterium Tuberculosis genome in a real time process using fluorescent probes which are called the molecular beacons. Results are obtained roughly in about 90 minutes, with minimum biohazard and a very little technical training for operation. This can even be set up easily in a simple laboratory without much technical requirements.

FIGURE.1. CBNAAT/GENEXPERT PROCEDURE.



The assessment of the diagnostic accuracy of X-pert MTB/RIF assay, found that when its used as an initial test without smear microscopy it had a pooled sensitivity of 89% and a specificity of 99%. However, when it was used for cases of negative smear microscopy the sensitivity was only 67% and specificity 99%[20].

In another clinical study, the sensitivity of the test on a single sputum sample was 92.2% for culture positive TB and 98.2% for both smear and culture positive patients and 72.5% for smear negative but culture positive cases, and there was a specificity of 99.2%. Sensitivity and specificity were slightly higher when three samples were tested[21]. CBNAAT was adopted in India by RNTCP in the year 2012. First it started as a pilot project in Maharashtra state, India. CBNAAT is currently available at more centers and the aim is to establish a link with all the peripheral hospitals to medical college hospitals and implemented in private set up.

A NOTE ON DRUG RESISTANCE IN TUBERCULOSIS

Drug resistance usually occurs because of point mutations occurring in the genome of Mycobacterium Tuberculosis. The Rifampicin resistance is associated with rpob gene mutation in 95% of cases. The resistance to Isoniazid, the mutations are mainly in the katG (50–95%) and inhA (up to 29 -45%) of genes. For Pyrazinamide resistance gene involved is the pncA gene (up to 98%) and to Ethambutol it is embB gene (50–65%). For the second line drugs, for Fluoroquinolone's mutation is in the gyrA–gyrB genes (75–95%) and for Aminoglycosides mainly in the rrs gene (up to 80%).

Because of these different gene involvements, there is no cross resistance among the commonly used drugs. The development of drug resistance in Tuberculosis is almost invariably because of monotherapy. In addition, the usage substandard quality drugs also can cause drug resistant Tuberculosis. Yet another, but the most

important factor is patient compliance to treatment which must be ensured for the prevention of a dreadful global burden- the drug resistant Tuberculosis.

Drug resistant TB can be either primary or acquired. Primary drug resistance is that is present at the time of starting the treatment. Secondary drug resistance occurs during the treatment, that is initially patient will be responsive, but due to several afore mentioned reasons, resistance develops. MDR TB: Multi Drug Resistant TB- Resistant to both Isoniazid and

Rifampicin XDR TB: Extremely drug resistant TB- MDR strains with drug resistance to any of Fluoroquinolones and to any of the three second line injectables like Amikacin, Kanamycin Capreomycin.

HIV/AIDS, PATHOGENESIS AND RELATION WITH TUBERCULOSIS

Human Immunodeficiency Virus is the etiologic agent of AIDS, it belongs to Human Retrovirus family and the Lentivirus subfamily. The hallmark of HIV and AIDS is a florid immunodeficiency resulting from a progressive both qualitative and quantitative deficiency of the subset of T lymphocytes, the helper cells. This occurs in setting of polyclonal immune system activation. These Helper T cells are defined phenotypically by the presence of CD4 molecule on its surface, which serves primarily as the cellular receptor for HIV.

The major co-receptors are CCR5 and CXCR4, for fusion and entry to cells, also serve as the primary receptors for chemo attractive cytokines known as chemokines. The mechanisms responsible for cellular depletion and dysfunction of CD4 T cells include

1. Direct infection and destruction by virus.
2. Indirect effects like immune clearance, cell death associated with abnormal immune activation, immune exhaustion etc.

Approximately one third of all deaths in AIDS are associated with Tuberculosis and it is the primary cause of death in about 15% of patients with HIV. In our country India, the prevalence of the disease is approximately 10% with HIV and active tuberculosis. Untreated TB will hasten the natural disease course of HIV.

Infection with atypical mycobacteria, the active TB develops early during HIV infection and can be considered as an early clinical sign of HIV disease. In one study, the median CD4 count at presentation of Tuberculosis was 326/μL^9. The clinical manifestations of TB in HIV patients are quite varied and show difference in radiological presentation with difference in CD4. Patients with relatively high CD4+ T cell counts, there will be typical pattern of pulmonary reactivation that is they present with fever, cough, difficulty in breathing, weight loss, evening rise of temperature, and a chest x ray with upper lobe apical cavities.

In patients with lower CD4 T counts, disseminated diseases are more common and chest x-ray reveals diffuse or lower lobe bilateral infiltrates consistent with miliary spread, pleural effusions, or hilar prominence due to lymphadenopathy. Some patients may have no symptoms, and screening for TB should be a part of the initial evaluation of all patients with HIV infection.

In the study conducted by R Dewan et al, eleven patients out of 100(11%) were positive by sputum microscopy for acid-fast bacilli and 40 (40%) were positive by CBNAAT. Thus, tuberculosis detection rate increased by more than three times using CBNAAT. There is a highly significant statistical difference in the diagnostic ability of CBNAAT when compared to sputum microscopy (the two-tailed p value is less than 0.001). CBNAAT positivity was seen across all ranges of CD4 counts and there was no statistically significant difference between CD4 count of CBNAAT positive and CBNAAT negative patients (p value = 0.264). CBNAAT also diagnosed

10 (25%) cases of rifampicin resistance among the 40 Mycobacterium tuberculosis positive cases. CBNAAT had 100% specificity for detection of rifampicin resistance[22].

In the study conducted by Gupta H et al, on 160 PTB/HIV patients. Out of 160 PTB/HIV patients, 116 (72.5%) were males and 44 (27.5%) were females. The mean age in this study was 38.01 yrs. and the sex ratio was 2.63:1 Middle aged 26-45 years (68.12%) individual were most affected especially the rural population (84.66%). Out of 24 sputum smear positive cases higher no. of sputum smear positive among in CD4 above 200 i.e., 19 (79.16%) and lower among CD4 below 200 i.e., 5 (20.83%). Among the CBNAAT positive cases who were resistant to Rifampicin, maximum 12 (67%) of them were previously treated cases followed by 6(33%) were new cases. By comparing CBNAAT and Sputum AFB positivity, CBNAAT positivity (45%) was found more than sputum smear microscopy (15%).CBNAAT positive and sputum AFB negative cases were 31.2%, while CBNAAT negative and sputum positive cases were minimum (1.2%), showing false negativity of sputum smear microscopy and high sensitivity and specificity of CBNAAT. Maximum cases with CD4 count >200, are both CBNAAT and Sputum AFB Negative and least cases were CBNAAT Negative and sputum positive in respect to CD4 count. It is found that 25% were resistant to rifampicin[23].

In the study conducted by the Department of Cardiothoracic Surgery, Government Mohan Kumaramangalam Medical College Hospital, Data collected were 150 HIV infected patients who tested sputum smear negative. Sputum samples were then sent for CBNAAT and sputum culture for mycobacteria. Female patients were 58% and males were 42%. Females were more in the study population when compared to males. Sensitivity of CBNAAT= 73.68%. Incidence rate as per

CBNAAT- 18.66%, Incidence rate as per sputum culture = 25.33%. They concluded study by reporting sputum microscopy is not a sensitive diagnostic tool for diagnosing pulmonary tuberculosis in people living with HIV. CBNAAT is a highly sensitive and specific tool for the diagnosis of TB, and it is highly useful for the early diagnosis of smear-negative TB in HIV infected patients[24].

In the study, conducted by Singh et al, performance of gene X-pert was compared to acid-fast microscopic examination using Ziehl-Neelsen (ZN) stain in patients with culture-confirmed tuberculosis. Out of total 914 specimens of clinically suspected patients of tuberculosis of all age groups, 683(75%) were pulmonary specimens and 231(25%) were extra-pulmonary. For pulmonary samples, the sensitivity and specificity for CBNAAT samples were 82.3%and98.5%while that for sputum smear were 63.7% and 99.3% respectively. The study concluded that development of the gene X-pert MTB/RIF assay is undoubtedly a landmark event in country like India as this test will help in rapid diagnosis of smear-negative and rifampicin resistant TB cases, which were earlier a challenge for the TB control program[25].

In another study, Boehme et al, conducted in patients from 5 sites in South Africa and Mumbai, India, patients suspecting pulmonary TB, HIV negative and positive were studied with sputum sample. The tests done were AFB microscopy, ZN method, culture in LJ media and CBNAAT. Among culture positive PTB, the sensitivity of the CBNAAT was 97.6%. For smear and culture positive patients, the sensitivity was 99.8, smear negative culture positive, it was 90.2%. The sensitivity with CBNAAT was compared between HIV positive (98.4%) and negative individuals (93.9%), but there were not statistically significant co relation[26].

MATERIALS AND METHODS

All retroviral positive patients with clinical suspicion of pulmonary tuberculosis including symptoms of cough with or without expectoration for more than two weeks, weight loss, fatigue, hemoptysis, and loss of appetite attending out-patient and in-patient department ofin Shri B M Patil hospital, Vijayapura, from November 2019 to April 2021 was considered forsputum analysis. Two sputum samples (one spot sample and other one early morning sample) was sent for NTEP laboratory for detection of AFB (acid fast bacilli) by method of ZN staining.

Those patients whose both sputum samples negative for AFB were considered as sputum negative retroviral positive patients and was considered for the present study. Those patient's sputum samples were sent for CBNAAT (Cartridge Based Nucleic Acid Amplification) analysis.

Usefulness of CBNAAT testing in sputum smear negative samples was assessed.

SOURCE OF DATA:

1. The study was among the patients with clinical suspicion of pulmonary tuberculosis, with HIV positive status attending OPD & IPD of BLDE hospital, Vijayapura.
2. The patients were informed about study in all respects and informed consent was obtained.
3. Period of study will be from NOVEMBER 2019 TO APRIL 2021.

Method of collection of Data (including sampling procedures if any):

Study type- cross- sectional study

Patients meeting the inclusion criteria were considered in the study

SAMPLE COLLECTION

Oral and written consent was taken from the subjects prior to the collection of specimens.

INCLUSION CRITERIA:

1. All retroviral positive patients with clinical suspicion of pulmonary tuberculosis
2. Patients whose sputum sample is negative for AFB

EXCLUSION CRITERIA:

Smear positive tuberculosis with HIV infection

Sample size calculation

- With Anticipated common Proportion of MTB by sputum microscopy 11% and by CBNAAT 40 % (27), the minimum sample size required was 94 patients with 95% power and 5% level of significance.

Formula used

- $n = \frac{(z\alpha + z\beta)\ 2\ 2\ p*q}{MD\ 2}$

  Where Z= Z statistic at a level of significance

  MD= Anticipated difference between two proportions

  P=Common Proportion

  q= 100-p

Statistical analysis:

1. Numerical variables are presented as Mean ±SD, and categorical variables are presented as frequency (%) and diagrams
2. Comparison of numerical variables between groups is done using unpaired t test/ Mann Whitney U test, and categorical variables by Mc nemars Chi square test.
3. Sensitivity, specificity, PPV and NPV is performed wherever required.

OBSERVATIONS AND RESULTS

A total of 94 patients with clinical suspicion of pulmonary tuberculosis were selected for thesis study. All 94 patients were retroviral positive and sputum negative patients. All 94 patients were on ART drugs. These 94-smear negative retroviral positive patients' sputum samples were subjected to CBNAAT analysis. Out of 94 smear negative samples, 13 samples were positive by CBNAAT analysis. Among those 13 samples, which were positive by CBNAAT analysis, one sample was rifampicin resistant.

TABLE.2. AGE DISTRIBUTION

Age (years)	Frequency	Percentage
<20	2	2.1
20-29	21	22.3
30-39	33	35.1
40-49	26	27.7
50-59	10	10.6
60+	2	2.1
Total	94	100.0

The above table depicts the age distribution of the patients in the study. The mean age of patients included in the study was 36.22. Ages of all the patients in the study were divided into six groups. First group was less than twenty years; second age group was between twenty and twenty-nine. Third age group was between thirty and thirty-nine; fourth group between forty and forty nine. The fifth group between fifty and fifty-nine: and the last sixth group was more than sixty years. Two (2.1%) patients were in group 1, twenty-one (22.3%) patients in group 2 and thirty-three

(35.1%) patients were in group 3, which was maximum. Twenty-six (27.7%) patients in group 4, ten (10.6%) patients in group 5 and two (2.1%) patients were in group 6.

FIGURE.2.AGE DISTRIBUTON

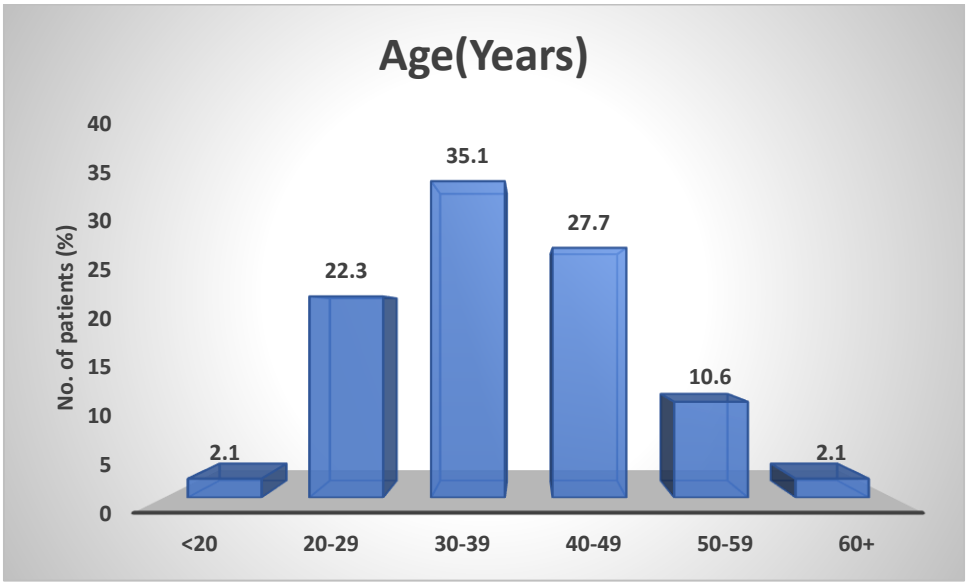


TABLE.3. GENDER DISTRIBUTION

GENDER	Frequency	Percentage
Female	51	54.3
Male	43	45.7
Total	94	100.0

Among 94 patients in the study, 51(54.3%) were females and 43(45.7%) were males. Our study observed female predominance.

FIGURE.3. GENDER DISTRIBUTION

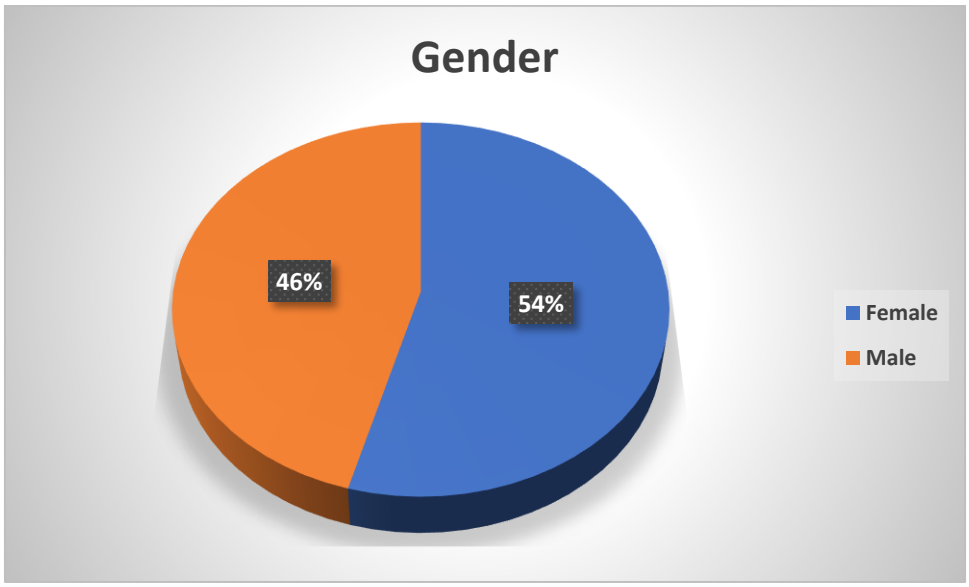


TABLE.4.CD4 COUNT DISTRIBUTION

CD4 count	Frequency	Percentage
<=100	13	13.8
101-200	24	25.5
201-300	22	23.4
301-400	16	17.0
401-500	15	16.0
501+	4	4.3
Total	94	100.0

Total of 94 PLHIV patients were included in the study. The mean CD4 count was 273.28. CD4counts of all 94 patients were divided into six groups. Cd4 counts less than 100 were under group 1, which had 13 patients (13.8%). Cd4 counts from 101 to 200 were under group 2, which had 24(25.5%) patients. Cd4 counts from 201 to 300 were under group 3, which had 22 (23.4%) patients. Cd4 counts, from 301 to 400 were under group 4, which had 16 (17 %) patients. Cd4 counts from 401 to 500 were under group 5, which had 15 (16 %) patients. Cd4 counts more than 500 were under group 6, which had 4 (4.3 %) patients.

FIGURE.4.CD4 COUNT DISTRIBUTION

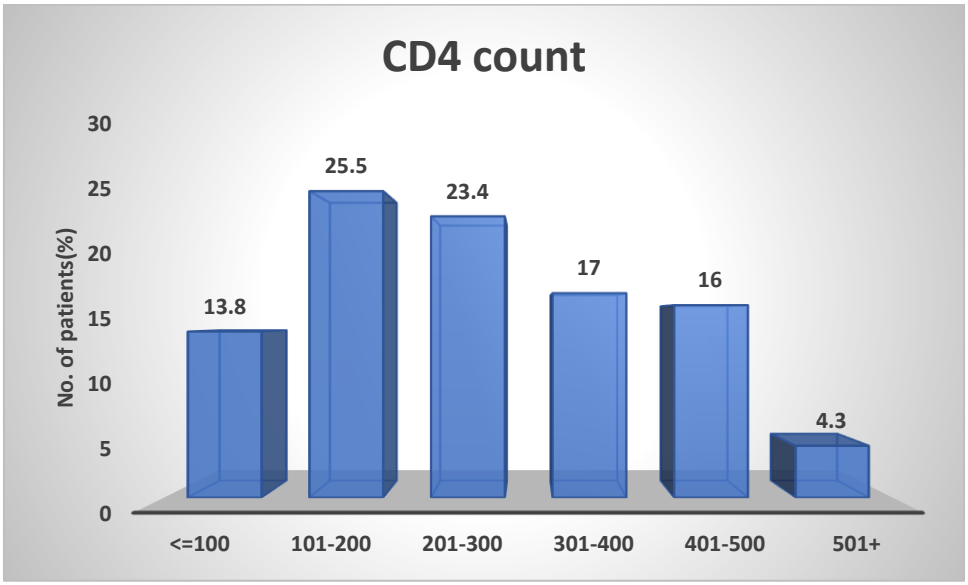


TABLE.5. CBNAAT ANALYSIS RESULT

CBNAAT	Frequency	Percentage
Negative	81	86.2
Positive	13	13.8
Total	94	100.0

CBNAAT study was done on all 94 (100%) PLHIV, whose sputum microscopy was negative, CBNAAT detected 13 (13.8%) positive cases among 94 cases which were negative by sputum studies. Rest 81(86.2 %) cases which were negative by sputum studies were also negative by CBNAAT study.

FIGURE.5. CBNAAT ANALYSIS RESULTS

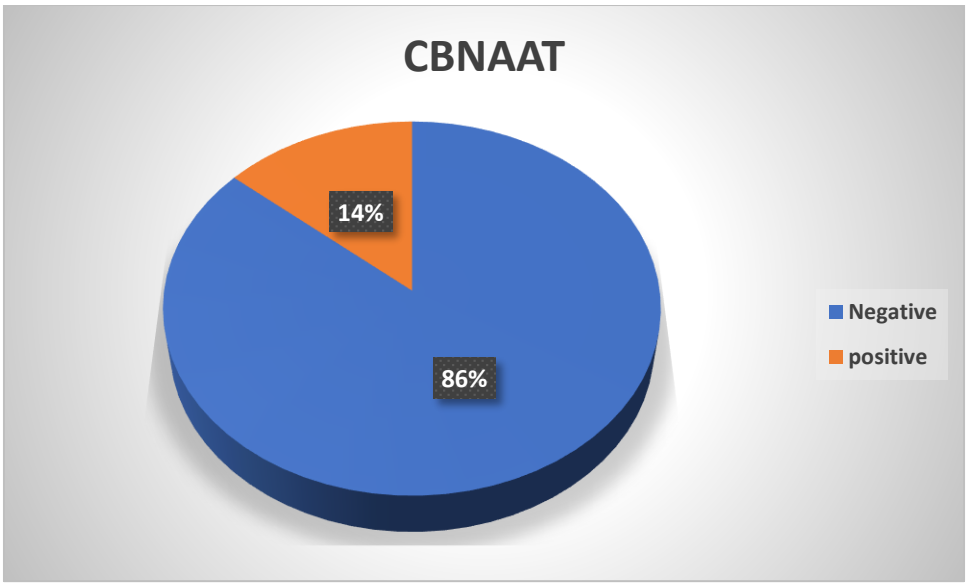


TABLE.6. RIFAMPICIN RESISTANCE

	Frequency	Percentage
NA	81	86.2
Resistance	1	1.1
Sensitive	12	12.8
Total	94	100.0

All 94 (100%) samples were subjected to CBNAAT analysis which yielded 13 positive cases. Among those 13 (13.8%) positive cases detected by CBNAAT study, one patient (1.1%) had rifampicin drug resistance. Other 12 (12.8%) cases were sensitive to rifampicin drug.

FIGURE.6. RIFAMPICIN RESISTANCE

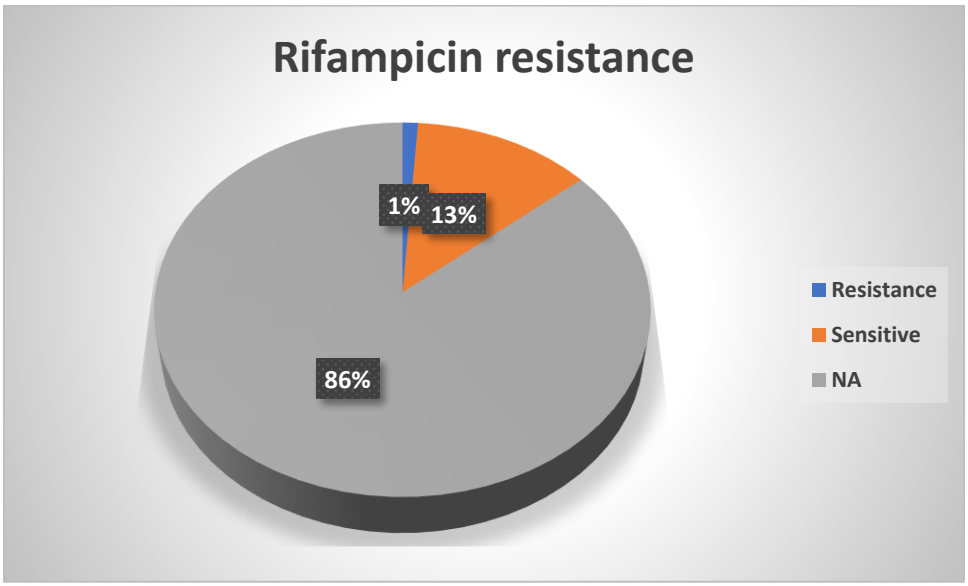


TABLE.7.BMI OF PATIENTS

BMI	Frequency	Percentage
<= 19.0000	60	63.8
19.0001+	34	36.2
Total	94	100.0

The mean BMI of the patients is 18.5. In total of 94 patients, 60 patients had BMI less than or equal to 19, 34 patients had BMI more than 19. Majority of the patients had low BMI, which was less than 19.

FIGURE.7.BMI OF PATIENTS

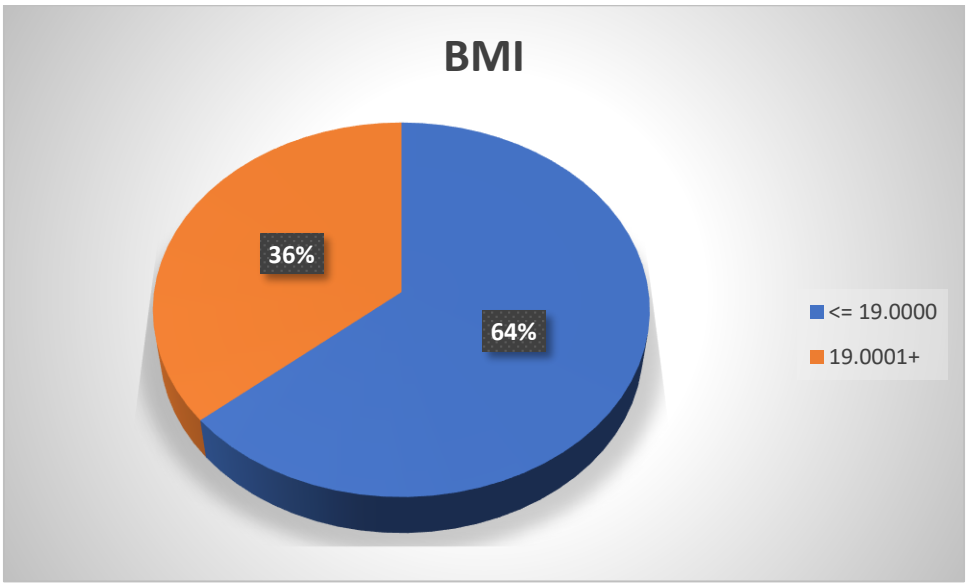


TABLE.8. Comparison of SPUTUM MICROSCOPY with CBNAAT in PTB-PLHIV patients

Sputum Microscopy	CBNAAT		Total
	Positive	Negative	
Positive	Nil	Nil	0
Negative	13	81	94

Total of 94 (100%) cases of PLHIV which were sputum microscopy negative was subjected to CBNAAT study. Out of 94 (100%) sputum microscopy cases, CBNAAT detected 13 (13.8%) positive cases. Rest 81 (86.2%) cases were CBNAAT and sputum microscopy negative.

FIGURE.8. Comparison of SPUTUM MICROSCOPY with CBNAAT in PTB-PLHIV patients

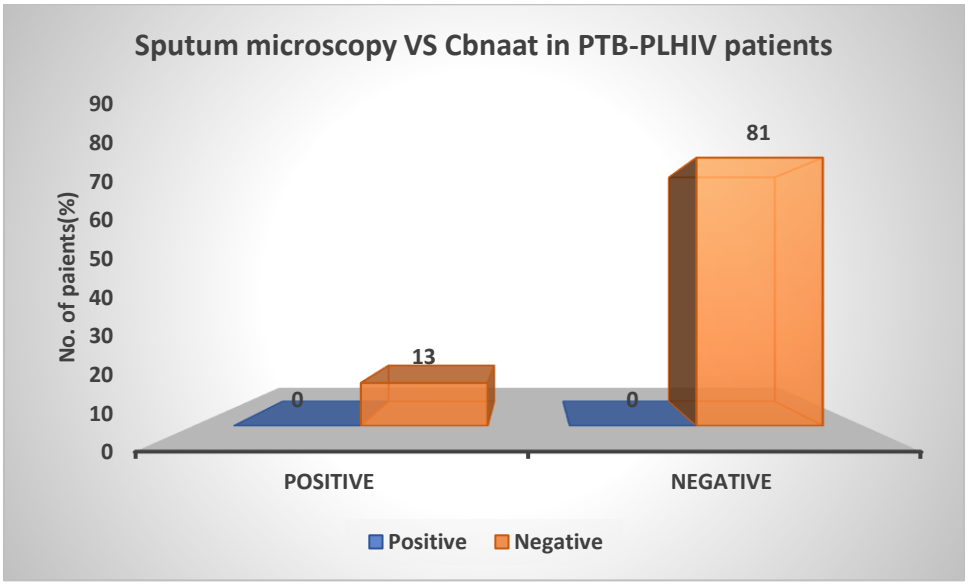


TABLE.9. Association of AGE with CD4 COUNT of patients

AGE (years)	CD4 COUNT						Total
	<=100	101-200	201-300	301-400	401-500	501+	
<20	0 0.0%	0 0.0%	1 4.5%	0 0.0%	1 6.7%	0 0.0%	2 2.1%
20-29	0 0.0%	4 16.7%	8 36.4%	3 18.8%	5 33.5%	1 25.0%	21 22.3%
30-39	1 7.7%	8 33.3%	7 31.8%	9 56.2%	5 33.3%	3 75.0%	33 35.1%
40-49	4 30.8%	10 41.7%	6 27.3%	2 12.5%	4 26.7%	0 0.0%	26 27.7%
50-59	6 46.2%	2 8.3%	0 0.0%	2 12.5%	0 0.0%	0 0.0%	10 10.6%
60+	2 15.4%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	2 2.1%
Total	13 100.0%	24 100.0%	22 100.0%	16 100.0%	15 100.0%	4 100.0%	94 100.0%

(chi-square value =52.7, p value =0.001*)

Above table depicts the association between age and CD4 count of the patients involved in the study. Maximum numbers (33 patients) were between age group 30 to 39 and among those maximum numbers (9 patients) had CD4 count between 301 to 400. Pearson Chi-square test value was 25.7 and p value was 0.001, which was statistically significant.

FIGURE.9. Association of AGE with CD4 COUNT of patients

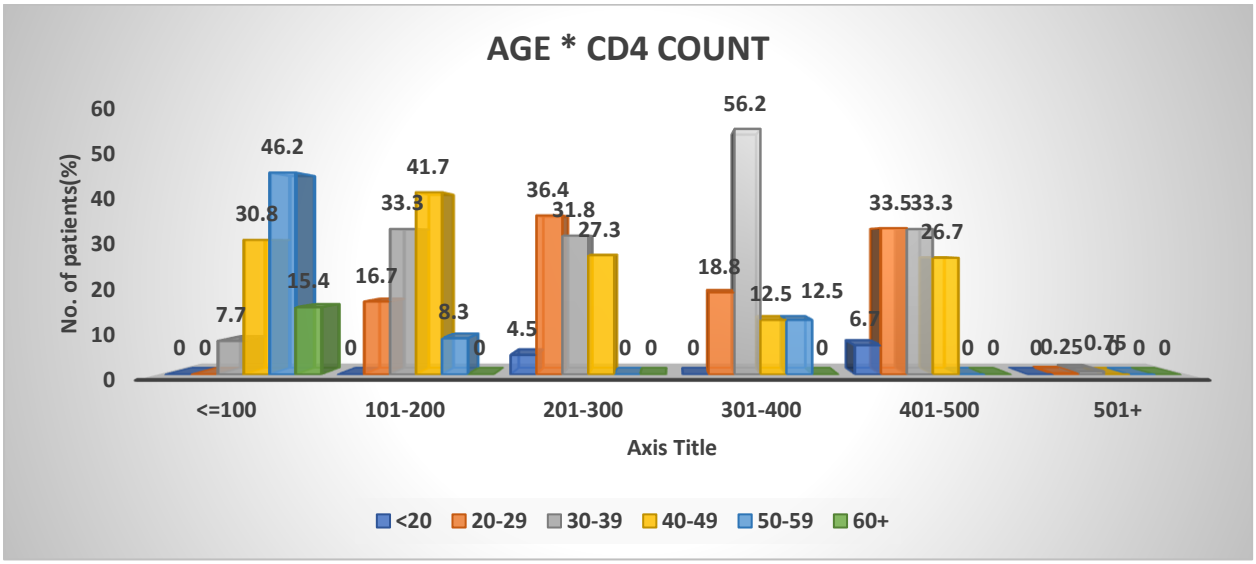


FIGURE.10. Association of CD4 COUNT with GENDER

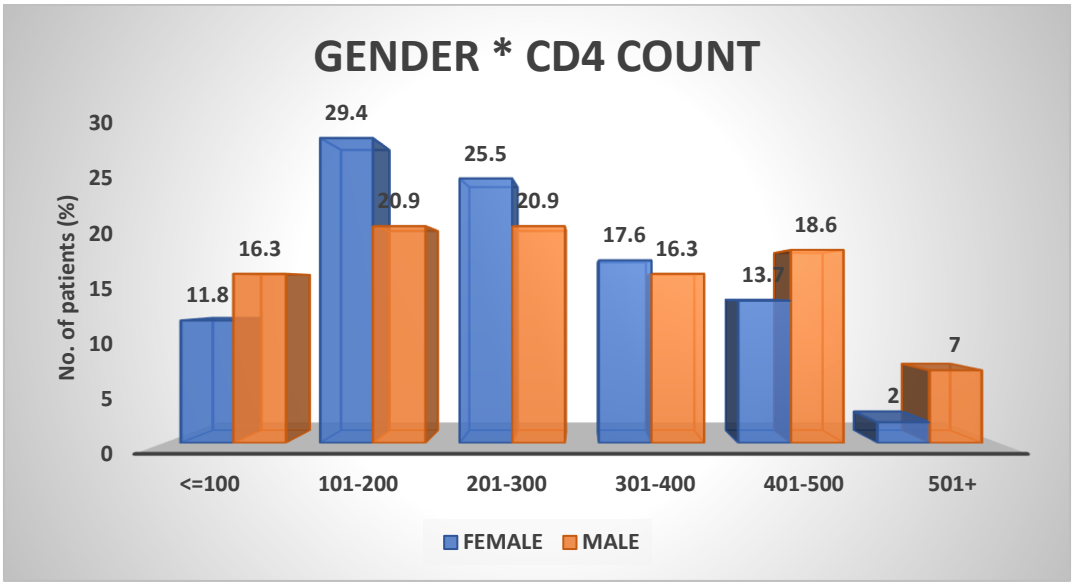


TABLE10.Association of CD4 COUNT with GENDER

CD4	GENDER		TOTAL
	FEMALE	MALE	
<=100	6 11.8%	7 16.3%	13 13.8%
101-200	15 29.4%	9 20.9%	24 25.5%
201-300	13 25.5%	9 20.9%	22 23.4%
301-400	9 17.6%	7 16.3%	16 17.0%
401-500	7 13.7%	8 18.6%	15 16.0%
501+	1 2.0%	3 7.0%	4 4.3%
TOTAL	51 100.0%	43 100.0%	94 100.0%

(chi-square value = 2.96, P value = 0.70)

The above table depicts the association of CD4 count of the patient with gender. In total of 94 patients, 13 (13.8%) had CD4 count less than 100, among those 13 patients; 6 (11.8%) patients were female and 7 (16.3%) were males. 24 (25.5%) patients had CD4 count between 101 to 200, in which 15 (29.4%) patients were females and 9 (20.9%) patients were males. 22 (23.4%) patients had CD4 count between 201 to 300, in which 13 (25.5%) patients were females and 9 (20.9%) patients were males. 16 (17.0%) patients had CD4 count between 301 to 400, in which 9 (17.6) patients were females and 7 (16.3%) patients were males. 15 (16.0%) patients had CD4 count between 401 to 500, in which 7 (13.3%) patients were females and 8 (18.6%) patients were males. 4 (4.3%) patients had CD4 count more than 500, in which 1 (2.0%) patient was female and 3 (7.0%) patients were males.

Maximum number (24 patients) had CD4 count between 101 to 200. Pearson Chi-square value was 2.96 and P value was 0.706, which not statistically significant.

TABLE.11. Association of CD4 COUNT with CBNAAT results

	CBNAAT		Total
	Negative	Positive	
<=100	12 14.8%	1 7.7%	13 13.8%
101-200	19 23.5%	5 38.5%	24 25.5%
201-300	20 24.7%	2 15.4%	22 23.4%
301-400	13 16.0%	3 23.1%	16 17.0%
401-500	15 18.5%	0 0.0%	15 16.0%
501+	2 2.5%	2 15.4%	4 4.3%
Total	81 100.0%	13 100.0%	94 100.0%

(chi-square value=8.937, p value= 0.0112)

The above depicts association of CD4 count with CBNAAT results. In total of 94 patients, 13 (13.8%) had CD4 count less than 100, among those 13 patients; 12 (14.8%) patients were CBNAAT negative and 1 (7.7%) patient was CBNAAT positive. 24 (25.5%) patients had CD4 count between 101 to 200, in which 19 (23.5%) patients were CBNAAT negative and 5 (38.5%) patients were CBNAAT positive. 22 (23.4%) patients had CD4 count between 201 to 300, in which 20 (24.7%) patients were CBNAAT negative and 2 (15.4%) patients were CBNAAT positive. 16 (17.0%) patients had CD4 count between 301 to 400, in which 13 (16.0%) patients were CBNAAT negative and 3 (23.1%) patients were CBNAAT

positive. 15 (16.0%) patients had CD4 count between 401 to 500, in which 15 (18.5%) patients were CBNAAT negative and no patient was CBNAAT positive. 4 (4.3%) patients had CD4 count more than 500, in which 2 (2.5%) patients were CBNAAT negative and 2 (15.4%) patients were CBNAAT positive. Pearson Chi-square value was 8.937 and P value was 0.112, which not statistically significant.

FIGURE.11. Association of CD4 COUNT with CBNAAT results

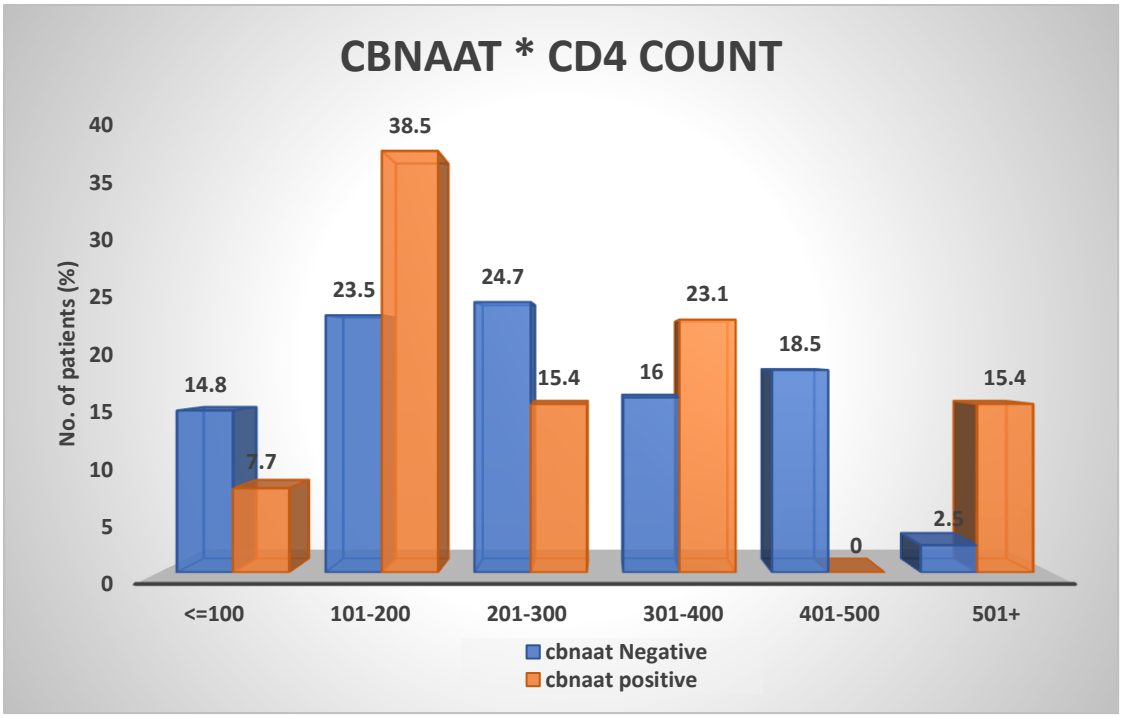


TABLE.12. Association of CD4 COUNT with Rifampicin resistance

	Rif resistance			Total
	NA	R	S	
<=100	12 14.8%	0 0.0%	1 8.3%	13 13.8%
101-200	19 23.5%	1 100.0%	4 33.3%	24 25.5%
201-300	20 24.7%	0 0.0%	2 16.7%	22 23.4%
301-400	13 16.0%	0 0.0%	3 25.0%	16 17.0%
401-500	15 18.5%	0 0.0%	0 0.0%	15 16.0%
501+	2 2.5%	0 0.0%	2 16.7%	4 4.3%
Total	81 100.0%	1 100.0%	12 100.0%	94 100.0%

(chi-square value = 11.6, p value= 0.310)

The above depicts the association of CD4 count with rifampicin resistance. In total of 94 (100%) patients in the study, 24 (25.5%) patients had CD4 count between 101 to 200. Among those 24 (25.5) patients, 5 were CBNAAT positive and 1 patient was CBNAAT negative. In those 5 patients with CD4 count between 101 and 200, one patient had rifampicin drug resistance detected by CBNAAT analysis. Pearson chi-square test value was 11.64 and P value was 0.310, which was statistically not significant.

FIGURE.12. Association of CD4 COUNT with Rifampicin resistance

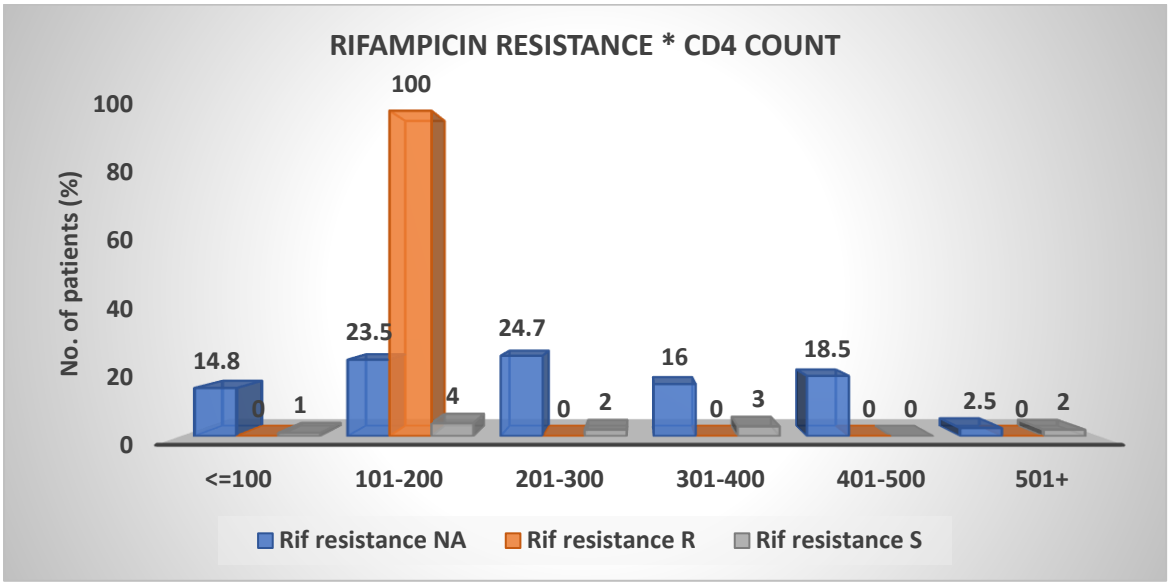


TABLE.13. **Association of AGE of the patient with CBNAAT results**

Age (years)	CBNAAT		Total
	Negative	Positive	
<20	2 2.5%	0 0.0%	2 2.1%
20-29	19 23.5%	2 15.4%	21 22.3%
30-39	27 33.3%	6 46.2%	33 35.1%
40-49	23 28.4%	3 23.1%	26 27.7%
50-59	8 9.9%	2 15.4%	10 10.6%
60+	2 2.5%	0 0.0%	2 2.1%
Total	81 100.0%	13 100.0%	94 100.0%

(chi-square value = 1.92 and P value = 0.859)

The above table represents the association of age of the patient with CBNAAT results. In total 94 (100.0%) patients, 2 (2.1%) patients were under 20 years of age; both patients were CBNAAT negative. 21 (22.3%) patients were between 20 to 29 age group, in which 19 (23.5%) patients were CBNAAT negative and 2 (15.4%) patients were CBNAAT positive. 33 (35.1%) patients were between 30 to 39 age group, in which 27 (33.3%) patients were CBNAAT negative and 6 (46.2%) patients were CBNAAT positive. 26 (27.7%) patients were between 40 to 49 age group, in which 23 (28.4%) patients were CBNAAT negative and 3 (23.1%) patients were CBNAAT positive. 10 (10.6%) patients were between 50 to 59 age group, in which 8 (9.9%) patients were CBNAAT negative and 2 (15.4%) patients were CBNAAT positive. 2 (2.1%) patients belonged to age group more than sixty and both were

CBNAAT negative. Maximum number of patients belonged to age group between 30 and 39, and maximum number of CBNAAT positive cases belonged to this age group.

FIGURE.13. Association of AGE of the patient with CBNAAT results

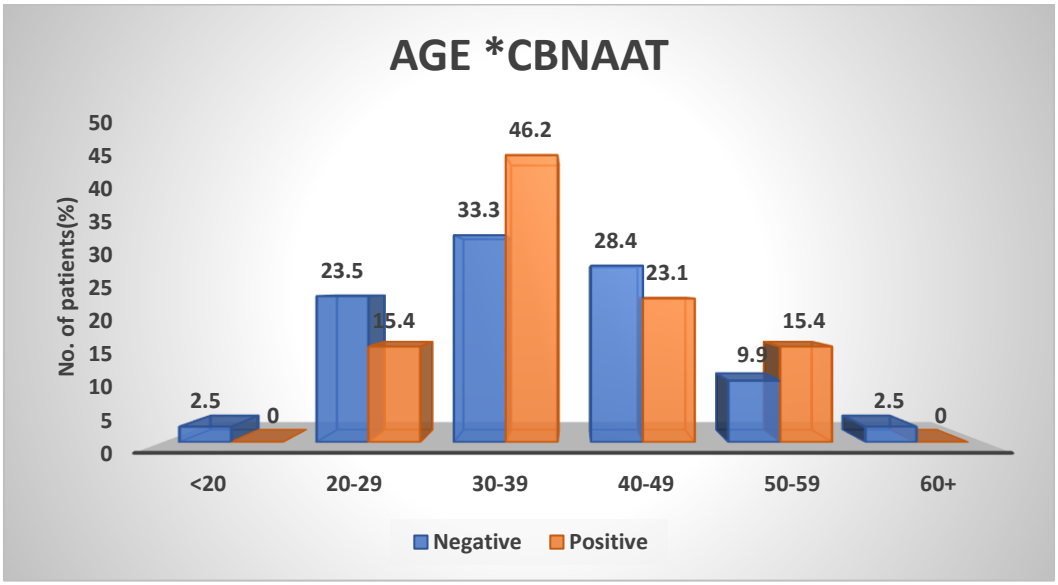


TABLE.14. Association of GENDER of the patient with CBNAAT results

GENDER	CBNAAT		total
	Negative	Positive	
Female	44 54.3%	7 53.8%	51 54.3%
Male	37 45.7%	6 46.2%	43 45.7%
Total	81 100.0%	13 100.0%	94 100.0%

(chi-square test value = 0.001 and P value = 0.975)

The above table represents the association of gender of the patient with CBNAAT results. In total of 94 (100.0%) patients in the study, 51 (54.3%) patients were females, who were maximum; 44 (54.3%) patients among them were CBNAAT negative and 7 (53.8%) patients were CBNAAT positive. 43 (45.7%) patients were males, 37 (45.7%) patients among them were CBNAAT negative and 6 (46.2%) patients were CBNAAT positive.

FIGURE.14. Association of GENDER of the patient with CBNAAT results

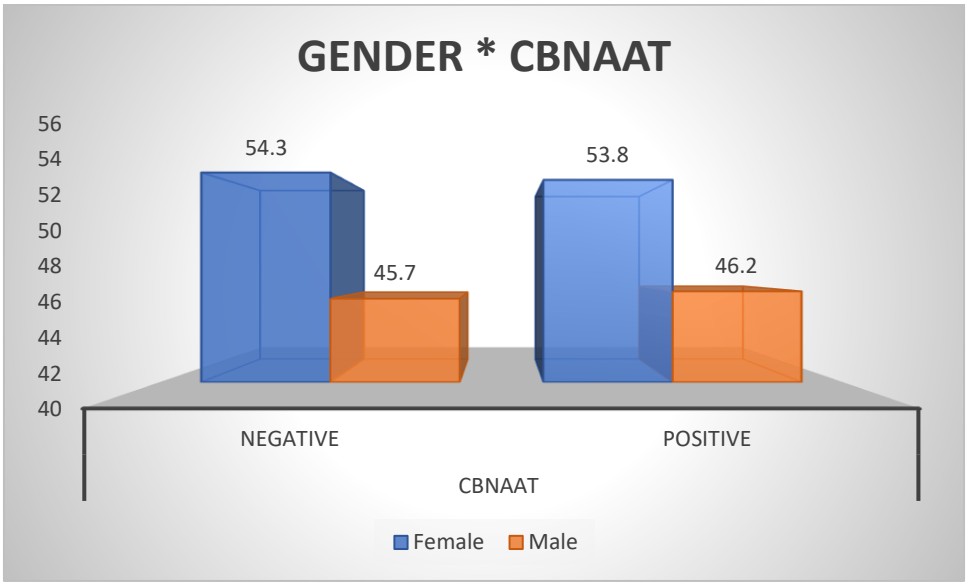


TABLE.**15. Association of Rifampicin Resistance with CBNAAT results**

Rifampicin Resistance	CBNAAT		Total
	Negative	Positive	
NA (not applicable)	81 100.0%	0 0.0%	81 86.2%
Resistance	0 0.0%	1 7.7%	1 1.1%
Sensitive	0 0.0%	12 92.3%	12 12.8%
Total	81 100.0%	13 100.0%	94 100.4%

(Chi square test value = 94.0, P value = 0.00)

The above table depicts the association of rifampicin resistance with CBNAAT results. In total of 94 patients in the study, 13 patients were CBNAAT positive and 81 were CBNAAT negative. Among those 13 (100.0%) positive patients, one patient had rifampicin drug resistance. Pearson chi-square test value was 94.0 and P value was 0.00, which was statistically significant.

FIGURE.15. Association of Rifampicin Resistance with CBNAAT results

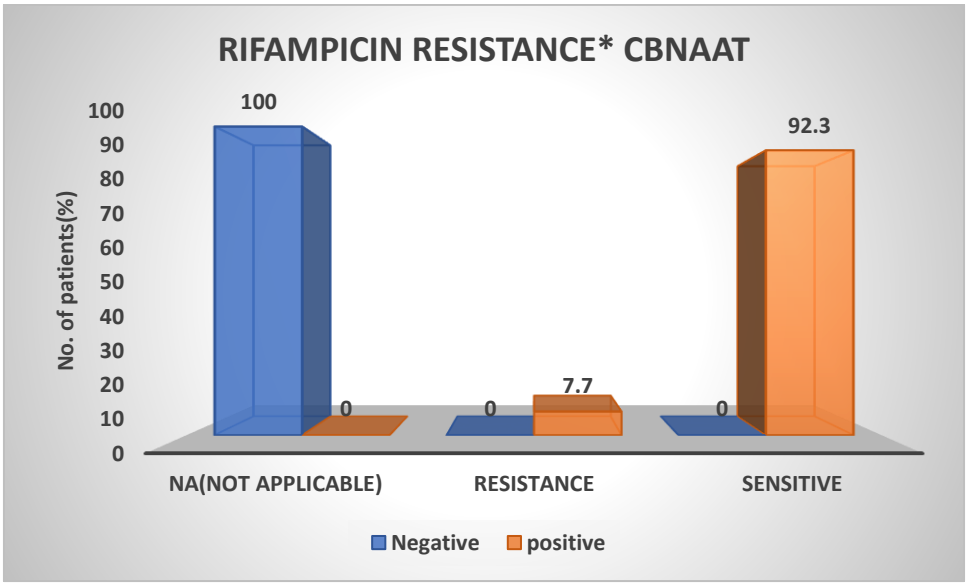


TABLE.16. Association of Rifampicin Resistance with GENDER

Rifampicin Resistance	GENDER		Total
	Female	Male	
NA (not applicable)	44 86.3%	37 86.0%	81 86.2%
Resistant	1 2.0%	0 0.0%	1 1.1%
Sensitive	6 11.8%	6 14.0%	12 12.8%
Total	51 100.0%	43 100.0%	94 100.0%

(chi-square test value = 0.931, p value = 0.628)

The above table depicts the association of rifampicin resistance with gender. In total of 94 patients in the study, 81 (86.2%) patients who were CBNAAT negative, 44 (86.3%) were females and 37 (86.0%) were males. Among 13 positive patients, 12 (12.8%) patients were rifampicin drug sensitive. Among those 12 patients, 6 (11.8%) patients were females and 6 (14.0%) were males. 1 (2.0%) patient among 13 positive cases, was resistant to rifampicin drug resistance who was a female. Pearson chi-square test value was 0.931 and P value was 0.628, which was statistically not significant.

FIGURE.16. Association of Rifampicin Resistance with GENDER

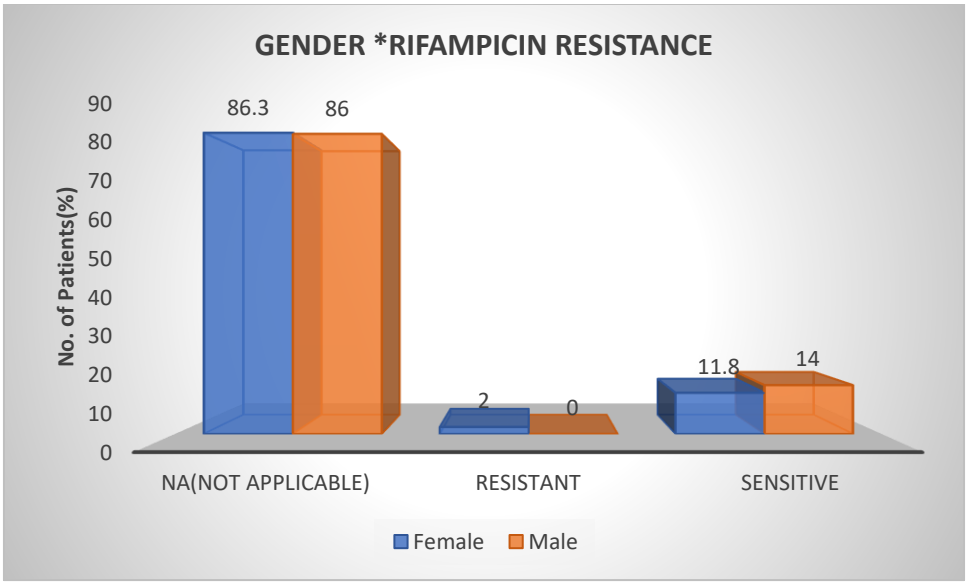


TABLE.17. Association if CD4 count with BMI

CD4 count	BMI		TOTAL
	<=19.0	19.01+	
<=100	12 20.0%	1 2.9%	13 13.8%
101-200	21 35.0%	3 8.8%	24 25.5%
201-300	13 21.7%	9 26.5%	22 23.4%
301-400	8 13.3%	8 23.5%	16 17.0%
401-500	5 8.3%	10 29.4%	15 16.0%
500+	1 1.7%	3 8.8%	4 4.3%
TOTAL	60 100.0%	34 100.0%	94 100.0%

(Chi square value 20.58, p value = .001*)

The above table depicts the association of CD4 count with BMI of the patients. In total of 94 (100%) patients in the study, 13 (13.8%) patients had CD4 count less than or equal to 100; among those 12 (20.0%) patients had BMI less than or equal to 19.0 and 1 (2.9%) patient had BMI more than 19. 24 (25.5%) patients had CD4 count between 101 to 200, among those 21 (35.0%) patients had BMI less than or equal to 19 and 3 (8.8%) patients had BMI more than 19. 22 (23.4%) patients had CD4 count between 201 to 300, among those 13 (21.7%) patients had BMI less than or equal to 19 and 9 (26.5%) patients had BMI more than 19. 16 (17.0%) patients had CD4 count between 301 to 400, among those 8 (13.3%) patients had BMI less than or equal to 19 and 8 (23.5%) patients had BMI more than 19. 15 (16.0%) patients had CD4 count between 401 to 500, among those; 5 (8.3%) patients had BMI less than or

equal to 19 and 10 (29.4%) patients had BMI more than 19. 4 (4.3%) patients had their CD4 count more than 500, among those; 1 (1.7%) had BMI less than or equal to 19 and 3 (8.8%) had BMI more than 19. Maximum number (24 patients) had their CD4 count between 101 to 200 and among them maximum (21 patients) had their BMI less than 19. Pearson Chi-square test value was 20.5 and P value was 0.001, which was statistically significant.

FIGURE.17. Association if CD4 count with BMI

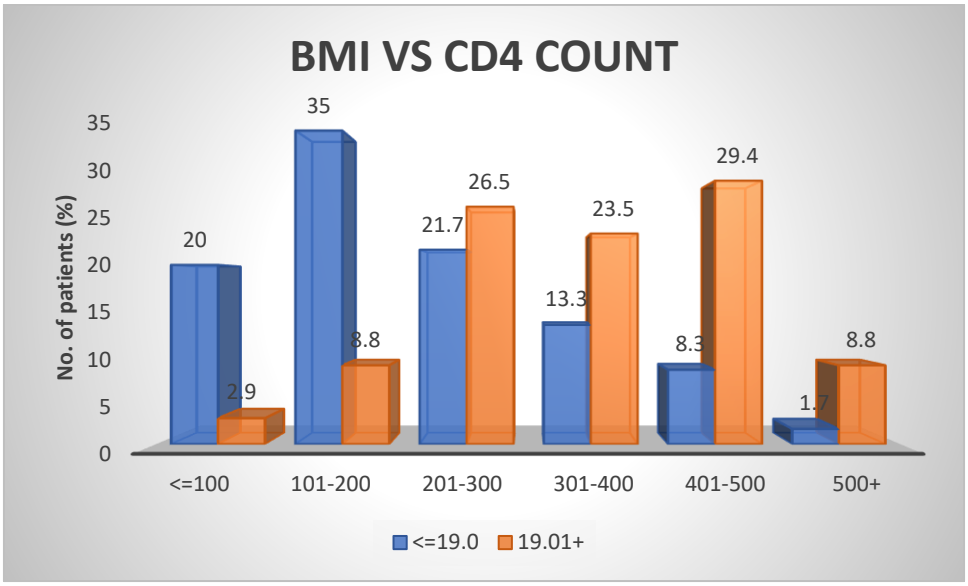


TABLE.18. Association of CBNAAT results with BMI of the patients

CBNAAT	BMI		TOTAL
	<=19.0	19.01+	
NEGATIVE	48 80.0%	33 97.1%	81 86.2%
POSITIVE	12 20.0%	1 2.9%	13 13.8%
TOTAL	60 100.0%	34 100.0%	94 100.0%

(Chi square value =5.2, p value =.021*)

The above table depicts the association of CBNAAT results with BMI of the patients. In total of 94 (100.0%) patients, 81 (86.2%) patients were CBNAAT negative, among them; 48 (80.0%) patients had their BMI less than or equal to 19 and 33 (97.1%) patients had BMI more than 19. 13 (13.8%) patients were CBNAAT positive, among them; 12 (20%) patients had BMI less than or equal to 19 and 1 (2.9%) patient had BMI more than 19. Among 13 CBNAAT positive cases, maximum number (12 patients) had BMI less than or equal to 19. Pearson chi-square test value was 5.2 and p value was 0.021, which was statistically significant.

FIGURE.18. Association of CBNAAT results with BMI of the patients

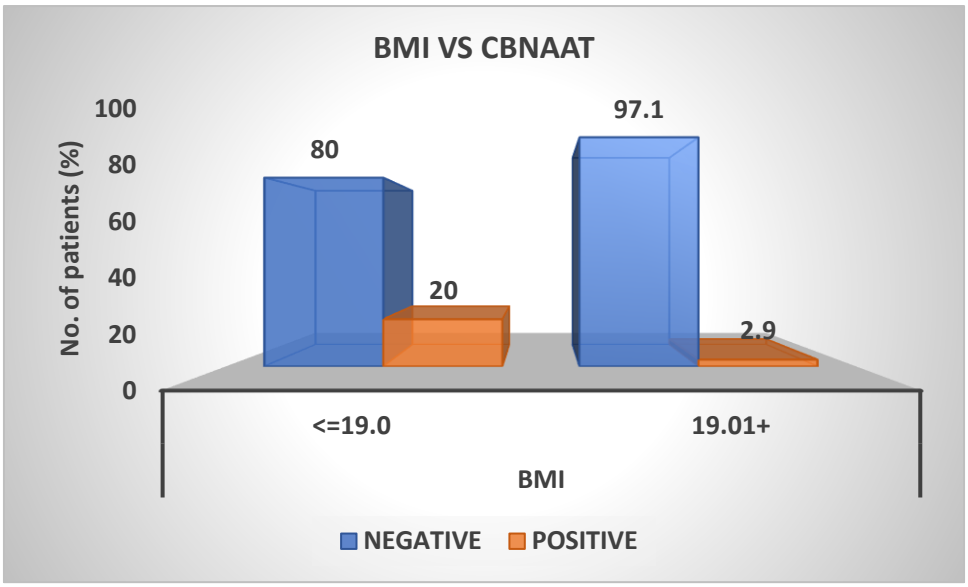


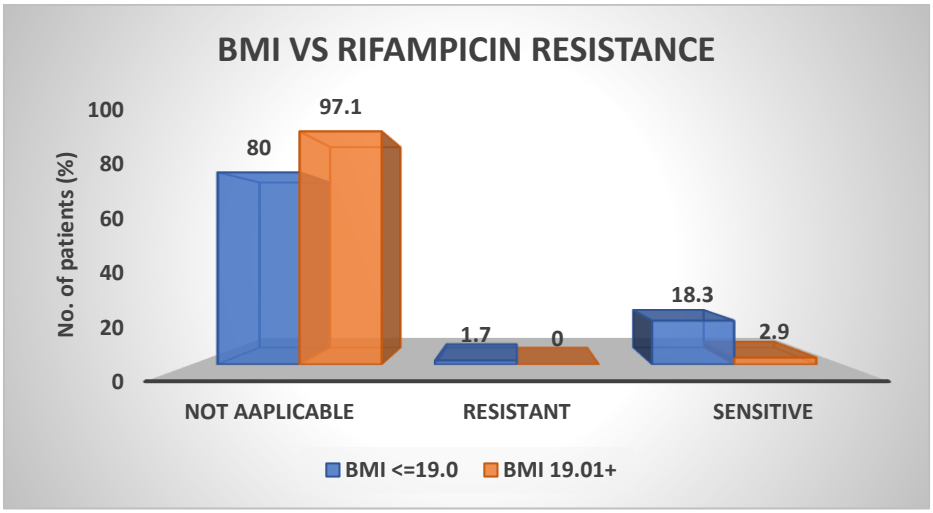
TABLE.19. Association of RIFAMPICIN RESISTANCE with BMI

RIFAMPICIN RESISTANCE	BMI		TOTAL
	<=19.0	19.01+	
NOT APPLICABLE	48 80.0%	33 97.1%	81 86.2%
RESISTANT	1 1.7%	0 0.0%	1 1.1%
SENSITIVE	11 18.3%	1 2.9%	12 12.8%
TOTAL	60 100.0%	34 100.0%	94 100.0%

(Chi square value = 5.327, p value =.070)

The above table depicts the association of rifampicin resistance with BMI of the patients. In total of 94 (100%) patients in the study, 13 patients were positive by CBNAAT study. Among those 13 patients, 12 (12.8%) patients were rifampicin drug sensitive. Maximum number of CBNAAT positive patients who were 11 (18.3%) were rifampicin sensitive had BMI less than or equal to 19 and 1 (2.9%) patient had BMI more than 19. 1 (1.1%) patient was rifampicin drug resistant whose BMI was less than 19. Pearson Chi-square test value was 5.32 and P value was 0.07, which was not statistically significant.

SFIGURE.19. Association of RIFAMPICIN RESISTANCE with BMI



DISCUSSION

This observational study was conducted over a period of one and half years from November2019 to April 2021 to analyse the utility of cartridge-based nucleic acid amplification test (CBNAAT) in early detection of pulmonary tuberculosis in sputum negative retroviral positive patients. A total of 94 patients included in this study and were analysed to study the CBNAAT effectiveness in comparison to sputum microscopy and its correlation with CD4 count.

Concomitant Human Immunodeficiency Virus (HIV) infection and Tuberculosis (TB) is a lamentable medical phenomenon with dreadful social and economic impact across the globe,

aptly described as a cursed duet. TB is the leading cause of death among people living with HIV, accounting for one in five HIV-related deaths. In India, it is estimated that 62% of HIV positive patients are affected with Tuberculosis (TB) so it is the most common Opportunistic Infection.

CD4 counts are critical in the control of infection with Mycobacterium tuberculosis, as quantitative and qualitative deficiency of these effector cells in HIV infected individuals increases the rate of both primary and reactivation of disease. Unlike other opportunistic infections which have a selective range of CD4 counts in which the disease occurs, TB occurs throughout the course of HIV[2].

In our study, the mean age group of patients was 36.22 years and most of the patients were between 30 and 39 age group: with range and SD. In a study by Kavya et al[2], most of the patients were of age group 31 to 50 years. In another study by Deewan et al[22], the mean age group was 35 years. In another study by Gupta H et al[23], most of the patients in age group between 36 and 45 years.

In our study, total 94 patients were included in the study, among them 51 were females and 43 were males; female preponderance was observed in our study. The gender ration observed in our study was like the study conducted by V Rajkumar et al[24], in which, a total of 150 were included in the study; among them 58% were female and 42% were males. In a study conducted by Deewan et al[22], a total of 100 patients were included in the study, among them 76% were males and 21% were females.

In our study, the mean CD4 count was 273. Maximum number of the patients in the study had CD4 count between 101 and 200. In a study by Deewan et al[22], their mean CD4 count was 230. HIV causes immunosuppression by direct depletion of host CD4+ T lymphocytes, which results in lymphocytopenia and down regulation of these immune cells. Vulnerability to TB diseases is increased in HIV positive patients. HIV infected patients with decreased CD4+ T cell count is associated with increased risk of TB, especially CD4+ T lymphocyte count less than 200 cells/mm3 is much more accompanied with higher TB incidence, which is observed in our study. Therefore, CD4+ T lymphocyte count remains the best predictor of a patient's immunological and clinical status, the risk of opportunistic infections like TB, and helps in diagnostic decision making, particularly for patients with advanced HIV disease. This could be partly due to impaired restoration of TB specific immunity when patients are severely immune compromised (baseline CD4+ T cell counts < 200 cells/mm3) at ART initiation and it might be because of primary infection or re-infection with the bacilli or re-activation of the existing latent TB because of severe immunosuppression[29].

The mean BMI of the patients in our study was 18.5. Low BMI is a risk factor for development of TB. In a study conducted by I Maro et al[28], low BMI and falling BMI were independently and significantly associated with increased risk of

developing TB. These observations suggest that malnutrition contributes to HIV-associated TB. Subjects with BMI < 17 kg/m2 or a 1-year BMI decrease of ≥0.5 kg/m2 have an increased risk of developing HIV-TB co infection. HIV-infected adults should be screened for TB disease. Risk of TB is even higher among PLHIV with low or falling BMI. While patients with low or falling BMI constitute the minority of subjects at risk for developing HIV-associated TB, recognizing the falling BMI may be a particularly useful predictor of TB risk among patients with a negative TST, in part because malnutrition can lead to TST anergy and thus thwart the identification of individuals who would benefit from INH preventive therapy. Low BMI is related to a greater bacillary burden during HIV-TB co-infection.

In our study, a total of 94 sputum negative samples with HIV positive status were subjected to CBNAAT analysis. CBNAAT detected 13 samples as positive. The detection rate of CBNAAT was higher than sputum microscopy testing. In a similar study done by V Rajkumar et al[24,] sensitivity of CBNAAT (82.3%) was more than sputum microscopy (63.7%). In another study by Gupta H et al[23], CBNAAT detection rate (45%) was higher than sputum microscopy (15%). In another study by Deewan et al[22], CBNAAT positivity (40%) more than sputum microscopy (11%). The incidence of TB in underdeveloped countries is increasing, and this is thought to be because of associated poor hygiene conditions and the greater prevalence of AIDS.

The global priorities for TB care and control are to improve case-detection and to detect cases earlier, including cases of smear- negative disease which are often associated with co-infection with the human immunodeficiency virus (HIV) and to enhance the capacity to diagnose multidrug-resistant tuberculosis (MDR-TB)[30]. These cases are often misdiagnosed due to the limitations of conventional diagnostic techniques.

Alarming increases in such cases, documented transmission, and rapid morbidity and mortality in these patients have highlighted the urgent need for rapid diagnostic methods. Microscopy and AFB analysis of sputum as well as non-respiratory samples has been a standard protocol for detection of tuberculosis. Due to low sensitivity and increased number of smear negative tuberculosis in HIV positive patients, it results in missing out many positive cases. In our study too, we observed that CBNAAT positivity rates were higher compared to sputum studies[30].

Each year, one among three people who are affected with TB are left undiagnosed or not noticed by health systems. These underdiagnosed or un-diagnosed 3.6 million people are main cause for high rates of TB transmission. The increase and more efficient execution of existing diagnostic tools will help countries to find and treat these millions of people. However, reaching the End TB Strategy targets needs a drastic increase in case detection which can be achieved only by major advances in novel diagnostic test technologies.

Culture, although extremely sensitive and specific system for TB diagnosis, needs two to eight -weeks to yield results and therefore alone does not assist in early diagnosis. CBNAAT is a molecular method of culture of Mycobacterium tuberculosis which also gives Rifampicin drug resistance in same sitting. It gives result within two hours. Unlike conventional nucleic acid amplification tests (NAATs), CBNAAT MTB/RIF is unique because sample processing and PCR amplification and detection are integrated into a single self-enclosed test unit, the CBNAAT Cartridge[31].

In the present study, among 13 CBNAAT positive cases, one case was detected as rifampicin resistant. In the study conducted by Srujana et al, out of 52 CBNAAT detected cases, 48 were rifampicin sensitive and 4 were rifampicin

resistant. In another study by Shilpa et al[33], among 137 CBNAAT positive cases, 12 were rifampicin drug resistant. The X-pert MTB/RIF (X-pert) assay is an automated nucleic acid amplification test that can detect both Mycobacterium tuberculosis and mutations associated with rifampicin resistance in the same sitting. PLHIV are also vulnerable to DR-TB infection. it also detects Rifampicin resistance as it targets the rpo-B gene of mycobacteria.

The emergence of multidrug-resistant Tuberculosis (MDRTB) and extensively drug-resistant TB (XDR-TB) in the past decade has highlighted the urgent need for both accurate diagnosis and Drug susceptibility testing. Approximately 3.6% of all new TB cases are caused by MDR strains, of which 10% are XDR-TB.33 CBNAAT can be a useful test for screening for MDR-TB. This is of reference to TB endemic areas like India where there is high prevalence of MDR-TB. The robustness of these data suggests that the test can be used in various resource scarce settings for detection and rapid decentralized screening of MDR in peripheral settings, including among patients with HIV is a break-through in tuberculosis care and control.

The WHO has projected 14 countries for high burden for MDR-TB and HIV co-infection. These are Angola, China, the Democratic Republic of the Congo, Ethiopia, India, Indonesia, Kenya, Mozambique, Myanmar, Nigeria, Papua New Guinea, South Africa, Thailand, and Zimbabwe. A few studies have estimated the burden of drug resistance among PLHIV ranging from 5.6% to 24% with genotypic tests, such as the cartridge-based nucleic acid–amplification test (CBNAAT), including automated Gene X-pert MTB/RIF assays and polymerase chain reaction–based line-probe assays (LiPAs)[33]. Failure of drug resistance leads to treatment failure and increases the spread of drug resistant strains in the community.

SUMMARY

Ninety-four patients with clinical suspicion of pulmonary tuberculosis with retroviral positive status was studied at B.L.D.E. U'S Shri. B.M. Patil Medical College Hospital and Research Centre, Vijayapura. The study was conducted during November 2019 to April 2019. This study was conducted to know the utility of CBNAAT in early detection of pulmonary tuberculosis in sputum negative retroviral positive patients in correlation with CD4 count.

1. The mean age group of patients was 36.22 years with range from 18 to 64 years. Most of the patients were between age group 30 and 39 years with standard deviation of 10.1.
2. Among 94 cases included in the study, 51 were females and 43 were males.
3. The mean CD4 count of patients included in the study was 273.3 cells per cubic millimetre of blood with range 67 to 560 cells/mm3. Most of the patients had CD4 count between 101 and 200.
4. The mean BMI of patients was 18.5 with SD of 1.35and range from 15 to 22.
5. 94 patients who were detected as negative by sputum microscopy were subjected for CBNAAT analysis which detected 13 positive cases and the rest 81 cases were detected as negative.
6. Among 13 positive samples detected by CBNAAT, one sample was rifampicin resistant.
7. There was statistical significance found between age and CD4 count with p value of 0.001 by chi-square testing.
8. There was statistical significance between rifampicin resistance and CBNAAT with p value of 0.00 by chi-square testing.

9. There was statistical significance between BMI and CD4 count with p value of 0.001 by chi-square testing. Most of the patients had low CD4 count between 101 and 200, also had BMI less than19.0.
10. There was statistical significance between BMI and CBNAAT with p value of 0.001 by chi-square testing. Most of the CBNAAT positive case (12 cases) had BMI less than 19.0.
11. There was no statistical significance between CD4 count and CBNAAT results by chi square testing. Lower CD4 count was observed in most of the CBNAAT positive cases.
12. There was no statistical significance between CD4 count and gender by chi square testing.
13. There was no statistical significance between CD4 count and rifampicin resistance by chi square testing. One sample which was rifampicin resistant had low CD4 count of 179 cells/mm3.
14. There was no statistical significance between age and CBNAAT results by chi square testing.
15. There was no statistical significance between gender and CBNAAT results by chi square testing. A total of 13 cases were detected by CBNAAT analysis, among them 7 were females and 6 were males.
16. There was no statistical significance between rifampicin resistance and gender by chi square testing. One sample was rifampicin resistant who was a female.
17. There was no statistical significance between rifampicin resistance and BMI by chi square testing. One sample was rifampicin resistant whose BMI was 16.5.

CONCLUSION

CBNAAT is the primary diagnostic test in PLHIV with clinical suspicion of tuberculosis. It has better detection rate than sputum microscopy, also it detects drug resistance to rifampicin which greatly impacts the treatment approach. CBNAAT can be used as screening tool for detection of MDR-TB. It plays vital role in early detection of mycobacterium tuberculosis which is important in reducing the infectivity and droplet spread and decreases the incidence of TB burden. CBNAAT reduces the false negative rates of sputum microscopy.

CBNAAT detects only mycobacterium species whereas sputum microscopy detects both mycobacterium and non -mycobacterium species, hence, reduces the false positive rates. Emphasis should be made on nutrition of the patient as it impacts greatly on the treatment outcomes and malnourished patients are prone for tuberculosis infection. Missing out many undiagnosed cases of Tuberculosis remains to be a global concern. The development of the X-pert MTB/RIF assay for the GeneXpert platform is considered as an important breakthrough in the fight against TB. Its high specificity and less time taking procedure makes it an excellent tool for timely diagnosis of such cases.

CBNAAT should be made primary testing modality for all presumptive TB cases even without HIV infection. It greatly reduces the incidence of MDR-TB and improves detection of drug resistance.

LIMITATIONS OF THE STUDY

1. Sample size included was low (94 cases).
2. The duration of study was smaller (November 2019 to April 2021).
3. Inadequate specimen varieties like extra-pulmonary samples.
4. Detection of mycobacterium was not confirmed with culture of the organism which is gold standard.
5. Rifampicin resistance not confirmed with LPA or culture.
6. The study was hospital based not community based.

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IEC/NCO - 131/2019
22-11-2019

B.L.D.E. (DEEMED TO BE UNIVERSITY)

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The ethical committee of this college met on 13-11-2019 at 3-15 pm to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: A study on utility of cartridge-based nucleic acide amplication test 9CBNAAT) in early detection of pulmonary tuberculosis in sputum negative retroviral positive patients

Name of PG student: Dr Nethra N, Department of General Medicine

Name of Guide/Co-investigator: Dr R M Honnutagi, Professor Department of General Medicine

DR RAGHVENDRA KULKARNI
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

INFORMED CONSENT FORM

TITLE OF RESEARCH:

‘‘A study on utility of Cartridge-based nucleic acid amplification test (CBNAAT) in early detection of pulmonary tuberculosis in sputum negative retroviral positive patients”

GUIDE : DR R.M. HONNUTAGI

P.G.STUDENT : DR NETHRA N

PURPOSE OF RESEARCH:

I have been informed that the purpose of this study is to access **“**A study on early detection of pulmonary tuberculosis in retroviral positive patients by using CBNAAT’’

PROCEDURE:

I understand that I will undergo detailed history and clinical examination and investigations.

RISKS AND DISCOMFORTS:

I understand that there is no risk involved in this study and I may experience mild pain during the above mentioned procedures.

BENEFITS:

I understand that my participation in this study will help to early Diagnose pulmonary tuberculosis in HIV infection

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulation of hospital. If the data is used for publication the identity will not be revealed

REQUEST FOR MORE INFORMATION:

I understand that I may ask for more information about the study at any time.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or withdraw from study at any time.

INJURY STATEMENT:

I understand in the unlikely event of injury to me during the study I will get medical treatment but no further medical compensation.

_____ _____

(Signature of Guardian) (Signature of patient)

STUDY SUBJECT CONSENT FORM:

I confirm that Dr. NETHRA N has explained to me the purpose of this research, the study procedure that I will undergo and the possible discomforts and benefits that I may experience, in my own language.

I have been explained all above in detail in my own language and I understand the same. I agree to give my consent to participate as a subject in this research project.

SIGNATURE OF PARTICIPANT DATE

SIGNATURE OF WITNESS DATE

PROFORMA

Name of the patient:

Age in years:

Sex:

Address:

Religion:

Occupation:

IP no/OP no:

Presenting Complaints:

Past history

Personal history:

1. Tobacco chewing

2. Smoking

3. Alcoholism

4. Diet- Veg/Mixed

5. No habits

Family history:

GENERAL PHYSICAL EXAMINATION:

Built:

Nourishment:

Ht(Cm):

Wt(Kg):

BMI:

Pallor

Icterus

Clubbing

Cyanosis

Lymphadenopathy

Edema

6. Vital parameters a. Pulse:

b. BP:

c. Respiratory rate:

d. Temperature

SYSTEMIC EXAMINATION:

RESPIRATORY SYSTEM

ABDOMEN EXAMINATION

CARDIOVASCULAR SYSTEM

CENTRAL NERVOUS SYSTEM

ECG:

NIKSHAY ID:

CD4 COUNT:

ART NUMBER:

CONCLUSION:

SIGNATURE

DATE:

MASTER CHART

sl no	age(yrs)	sex	ip no	nikshay id	CD4	BMI	cbnaat	rif resistance
1	40	m	5803	13086069	212	19	negative	S
2	50	m	10763	14030301	88	16.2	negative	S
3	34	m	13032	14271047	480	20	negative	S
4	42	m	13639	14318581	240	18.2	negative	S
5	36	m	13906	13976651	532	19.6	negative	S
6	39	m	142777	14436034	324	18.3	negative	S
7	42	m	14713	14466785	490	18.2	negative	S
8	55	m	15768	14632369	340	17.7	**positive**	S
9	40	f	2894	15810466	156	17.2	negative	S
10	51	m	2945	19048175	67	15	negative	S
11	40	f	42238	12321403	372	18.6	negative	S
12	32	f	57863	10765226	289	19	negative	S
13	52	f	28784	10499691	89	16.2	negative	S
14	38	m	69872	9476142	298	18.2	negative	S
15	45	f	21180	9268012	88	17.9	negative	S
16	45	m	5872	9087728	268	18.1	negative	S
17	35	f	115875	8222149	182	18.2	negative	S
18	25	m	32554	7664059	486	19.1	negative	S
19	48	m	58745	7103924	112	17.7	negative	S
20	30	m	5875	6967546	332	19.5	negative	S
21	32	m	254789	6992628	165	18.9	negative	S
22	38	f	25878	552218	314	19.6	negative	S
23	60	m	25458	5854478	98	16.2	negative	S
24	18	m	3568	4593246	267	19.3	negative	S
25	42	m	26578	5735020	198	20.3	negative	S
26	50	m	854	4586932	156	19	negative	S
27	32	m	89276	12936454	402	20.1	negative	S
28	21	m	89277	702228772	512	17.6	**positive**	S
29	32	f	89279	12937036	364	18.3	**positive**	S
30	34	f	89280	12958730	190	17.9	**positive**	S
31	40	f	89281	12958582	256	18.6	**positive**	S
32	32	f	89320	13003917	144	18.5	negative	S
33	40	f	89360	13383220	167	18.9	**positive**	S
34	20	f	89797	13366633	468	20	negative	S

35	35	m	89771	13358170	502	22	**positive**	S
36	28	f	89803	13367135	209	19.3	negative	S
37	20	f	89807	1336828	268	18	negative	S
38	20	m	89809	13358883	437	18.9	negative	S
39	40	f	89876	13359062	168	20.7	negative	S
40	36	m	89881	13359102	78	20.2	negative	S
41	40	f	89882	13359152	193	20.4	negative	S
42	33	f	89931	13339276	452	19.3	negative	S
43	50	f	89934	13354802	84	16.1	negative	S
44	48	f	89938	13355847	147	17.7	negative	S
45	35	f	89946	10563742	280	18.6	negative	S
46	25	f	90059	13356407	254	19.8	negative	S
47	55	f	90068	13523603	328	19.9	negative	S
48	34	m	110069	13346575	340	20.1	negative	S
49	45	m	102673	13358952	178	16.7	negative	S
50	28	m	102676	13359035	260	17.7	negative	S
51	30	m	102681	13381954	460	18.8	negative	S
52	35	m	102683	13371583	186	18.2	negative	S
53	22	f	102711	13430289	450	19	negative	S
54	21	m	102777	13367981	390	17.4	negative	S
55	21	f	102799	13430781	280	17.6	negative	S
56	30	f	109082	13430828	264	18.2	negative	S
57	29	m	109120	13430902	298	19.2	negative	S
58	23	f	109022	13430954	328	18.4	negative	S
59	42	m	109138	13430996	88	16.2	negative	S
60	26	f	109140	13431042	350	17.6	negative	S
61	24	f	109142	13431083	180	18.2	negative	S
62	50	f	109153	13431563	98	16.8	negative	S
63	25	f	109171	10512240	286	20.2	negative	S
64	28	m	109177	13431249	298	19.6	negative	S
65	32	f	109178	13439352	380	19.8	negative	S
66	40	m	109220	12321140	485	17.6	negative	S
67	48	m	109242	13439524	100	17.8	negative	S
68	40	f	109243	13439646	450	19.6	negative	S
69	28	f	109254	13439761	480	19.6	negative	S
70	32	f	109257	13440328	189	18	**positive**	S
71	44	f	109263	13464726	260	19.6	negative	S
72	65	f	109266	13464839	96	18	negative	S
73	21	f	109270	13466217	170	17.9	negative	S
74	30	m	109271	13466466	176	16.2	negative	S
75	35	f	109359	13113117	212	17.5	**positive**	S
76	40	f	109392	13543802	312	19.8	negative	S

77	44	f	109398	13543963	78	16.1	negative	S
78	18	f	109400	12829802	480	21	negative	S
79	42	m	109401	12791937	258	18.6	negative	S
80	30	m	109497	8382420	340	18.2	**positive**	S
81	37	f	109511	12742677	560	22	negative	S
82	50	f	109514	13622234	190	18.6	negative	S
83	35	m	109515	13622624	460	19.6	negative	S
84	24	m	109516	13589030	197	19	negative	S
85	32	m	109518	12779515	352	19.6	negative	S
86	45	f	109524	13555392	485	20	negative	S
87	30	f	103525	12467449	294	19.3	negative	S
88	48	f	109526	12467555	186	18.2	negative	S
89	36	f	109532	12467590	360	19.6	negative	S
90	35	f	109535	12467685	198	16.3	negative	S
91	37	f	109542	12368701	250	19.3	negative	S
92	23	f	109543	12369933	179	16.3	**positive**	**R**
93	50	m	109550	12468122	96	16.1	**positive**	S
94	46	m	198754	12746859	136	17	**positive**	s
mean	36.2234043				273.28723	18.5329787		